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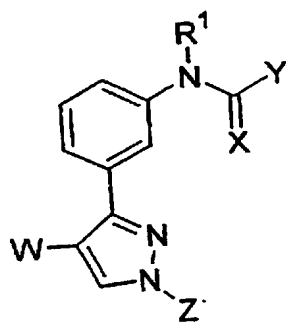
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(54) Title: PYRAZOLE DERIVATIVES WHICH MODULATE HUMAN SEROTONIN RECEPTORS



(A)

(57) Abstract: Disclosed herein are non-endogenous, constitutively activated forms of the human 5-HT_{2A} and human 5-HT_{2C} receptors and uses of such receptors to screen candidate compounds. Further disclosed herein are candidate compounds identified by the screening method which act at the 5HT_{2A} receptors. Yet further disclosed is a new class of compounds (I) which act at the 5HT_{2A} receptors. This new class encompasses the candidate compounds. In Formula (A) W is F, Cl, Br, I, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl; X is O or S or NR²; Y is NR³R⁴, or (CH₂)_mR⁵, or O(CH₂)_nR⁶; m is an integer between 0 and 4, inclusive; n is an integer

between 0 and 4, inclusive; Z is H, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl; R¹, R², R³ and R¹⁰ are as defined in the application.



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PYRAZOLE DERIVATIVES WHICH MODULATE HUMAN SEROTONIN RECEPTORS

5 The benefit of U.S. Provisional Number 60/152,708, filed September 7, 1999, U.S. Provisional Number 60/112,909, filed December 18, 1998, U.S. Provisional Number 60/123,000 filed March 5, 1999, U.S. Serial Number 09/292,071, U.S. Serial Number 09/292,072 and 09/292,069, all filed April 14, 1999, is hereby claimed.

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FIELD OF THE INVENTION

 The present invention relates to small molecule modulators of non-endogenous, constitutively active serotonin receptors thereof; preferably, the small molecule modulators are preferentially selective for the human 5HT_{2A} receptor over the human 5HT_{2C} receptor, and most preferably, the small molecule modulators are inverse agonists
15 to the 5HT_{2A} receptor.

BACKGROUND OF THE INVENTION**I. G protein-coupled receptors**

 G protein-coupled receptors share a common structural motif. All these receptors
20 have seven sequences of between 22 to 24 hydrophobic amino acids that form seven alpha helices, each of which spans the membrane. The transmembrane helices are joined by strands of amino acids having a larger loop between the fourth and fifth transmembrane helix on the extracellular side of the membrane. Another larger loop, composed primarily of hydrophilic amino acids, joins transmembrane helices five and six on the intracellular side of the
25 membrane. The carboxy terminus of the receptor lies intracellularly with the amino terminus in the extracellular space. It is thought that the loop joining helices five and six, as well as, the carboxy terminus, interact with the G protein. Currently, Gq, Gs, Gi and Go are G proteins that have been identified. The general structure of G protein-coupled receptors is shown in Figure 1.

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 Under physiological conditions, G protein-coupled receptors exist in the cell membrane in equilibrium between two different states or conformations: an "inactive" state and an "active" state. As shown schematically in Figure 2, a receptor in an inactive state is unable to link to the intracellular transduction pathway to produce a biological response.

Changing the receptor conformation to the active state allows linkage to the transduction pathway and produces a biological response.

A receptor may be stabilized in an active state by an endogenous ligand or an exogenous agonist ligand. Recent discoveries such as, including but not exclusively limited to, modifications to the amino acid sequence of the receptor provide means other than ligands to stabilize the active state conformation. These means effectively stabilize the receptor in an active state by simulating the effect of a ligand binding to the receptor. Stabilization by such ligand-independent means is termed "constitutive receptor activation."

II. Serotonin receptors

Receptors for serotonin (5-hydroxytryptamine, 5-HT) are an important class of G protein-coupled receptors. Serotonin is thought to play a role in processes related to learning and memory, sleep, thermoregulation, mood, motor activity, pain, sexual and aggressive behaviors, appetite, neurodegenerative regulation, and biological rhythms. Not surprisingly, serotonin is linked to pathophysiological conditions such as anxiety, depression, obsessive-compulsive disorders, schizophrenia, suicide, autism, migraine, emesis, alcoholism, and neurodegenerative disorders. With respect to on anti-psychotic treatment approaches focused on the serotonin receptors, these types of therapeutics can generally be divided into two classes, the "typical" and the "atypical." Both have anti-psychotic effects, but the typicals also include concomitant motor-related side effects (extra pyramidal syndromes, *e.g.*, lip-smacking, tongue darting, locomotor movement, etc). Such side effects are thought to be associated with the compounds interacting with other receptors, such as the human dopamine D2 receptor in the nigro-striatal pathway. Therefore, an atypical treatment is preferred. Haloperidol is considered a typical anti-psychotic, and clozapine is considered an atypical anti-psychotic.

Serotonin receptors are divided into seven subfamilies, referred to as 5-HT1 through 5-HT7, inclusive. These subfamilies are further divided into subtypes. For example, the 5-HT2 subfamily is divided into three receptor subtypes: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}. The human 5-HT_{2C} receptor was first isolated and cloned in 1987, and the human 5-HT_{2A} receptor was first isolated and cloned in 1990. These two receptors are thought to be the site of action of hallucinogenic drugs. Additionally, antagonists to the 5-HT_{2A} and 5-HT_{2C} receptors are believed to be useful in treating depression, anxiety, psychosis, and eating disorders.

U.S. Patent Number 4,985,352 describes the isolation, characterization, and expression of a functional cDNA clone encoding the entire human 5-HT_{1C} receptor (now

known as the 5-HT_{2C} receptor). U.S. Patent Number 5,661,012 describes the isolation, characterization, and expression of a functional cDNA clone encoding the entire human 5-HT_{2A} receptor.

5 Mutations of the endogenous forms of the rat 5-HT_{2A} and rat 5-HT_{2C} receptors have been reported to lead to constitutive activation of these receptors (5-HT_{2A}: Casey, C. *et al.* (1996) *Society for Neuroscience Abstracts*, 22:699.10, hereinafter "Casey"; 5-HT_{2C}: Herrick-Davis, K., and Teitler, M. (1996) *Society for Neuroscience Abstracts*, 22:699.18, hereinafter "Herrick-Davis 1"; and Herrick-Davis, K. *et al.* (1997) *J. Neurochemistry* 69(3): 1138, hereinafter "Herrick-Davis-2"). Casey describes a mutation of the cysteine residue at
10 position 322 of the rat 5-HT_{2A} receptor to lysine (C322K), glutamine (C322Q), and arginine (C322R) which reportedly led to constitutive activation. Herrick-Davis 1 and Herrick-Davis 2 describe mutations of the serine residue at position 312 of the rat 5-HT_{2C} receptor to phenylalanine (S312F) and lysine (S312K), which reportedly led to constitutive activation.

15

SUMMARY OF THE INVENTION

The present invention relates to small molecule modulators of non-endogenous, constitutively activated forms of the human 5-HT_{2A} and human 5-HT_{2C} receptors that are, preferably, preferentially selective for the 5-HT_{2A} receptor, and most preferably, have inverse agonist characteristics at the receptor.

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BRIEF DESCRIPTION OF THE DRAWINGS

In the following figures, bold typeface indicates the location of the mutation in the non-endogenous, constitutively activated receptor relative to the corresponding endogenous receptor.

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Figure 1 shows a generalized structure of a G protein-coupled receptor with the numbers assigned to the transmembrane helices, the intracellular loops, and the extracellular loops.

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Figure 2 schematically shows the active and inactive states for a typical G protein-coupled receptor and the linkage of the active state to the second messenger transduction pathway.

Figure 3 provides a graphic summary of dose-response results from *in vivo* analysis of compound 116082 in a 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ("DOI") inhibition study (fine movement; ambulation; and animal rearing outcome

measures). 116082 was administered 30 min prior to DOI. Animals were placed in locomotor activity cages 10 min after DOI administration and activity was measured for 10 min. Results are presented as total activity counts over the 10 min of exposure to the locomotor activity apparatus.

5 **Figure 4** provides a graphic summary of dose-response results from *in vivo* analysis of compound 116081 in a DOI inhibition study (fine movement; ambulation; and animal rearing outcome measures). 116081 was administered 30 min prior to DOI. Animals were placed in locomotor activity cages 10 min after DOI administration and activity was measured for 10 min. Results are presented as total activity counts over the
10 10 min of exposure to the locomotor activity apparatus.

Figure 5 provides a graphic summary of results from *in vivo* analysis of compound 116082 in a catalepsy-muscle rigidity study. 116082 (AR10, AR40, and AR80 mg/kg) and haloperidol (Hal.0.8 mg/kg) were compared in the bar catalepsy test in rats. The test compounds were administered intraperitoneally 60 min prior to the test. Time during
15 which both forelimbs remained on the bar was recorded up to a maximum of 30 sec. Values represent the average of 3 consecutive measurements (means \pm SEMs, n = 6-8/group). *P<0.05 vs. vehicle, Student t-test.

Figure 6 provides a graphic summary of the effects of oral administration of 116082 and 116081 in a DOI inhibition study in rats. Values represent the mean \pm SEMs
20 (n = 6/group). #P<0.05 vs. vehicle/vehicle, *P<0.05 vs. vehicle/DOI, Student t-test.

Figure 7 provides a graphic summary of the reversal of MK-801-induced hyperactivity using 116081 (7A) and clozapine (7B). 116081 and clozapine dose-dependently attenuated MK801-induced hyperactivity as measured by a decrease in ambulations in MK-801-treated animals. 116081 produced a significant reversal of the
25 effect of MK-801 at a dose of 25 μ mol/kg (10 mg/kg). Results are presented as total activity counts over the 120 min of exposure to the locomotor activity apparatus after administration of MK-801. Data (mean \pm SEM) were analyzed by ANOVA followed by Dunnett's test (n=6-8/group).

Figure 8 is a representation of the preferred vector, pCMV, used herein.

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DEFINITIONS

The scientific literature that has evolved around receptors has adopted a number of terms to refer to ligands having various effects on receptors. For clarity and consistency, the following definitions will be used throughout this patent document. To the extent that these definitions conflict with other definitions for these terms, the following definitions shall control.

AGONISTS shall mean moieties that activate the intracellular response when they bind to the receptor, or enhance GTP binding to membranes.

AMINO ACID ABBREVIATIONS used herein are set out in Table 1:

| TABLE 1 | | |
|----------------|-----|---|
| ALANINE | ALA | A |
| ARGININE | ARG | R |
| ASPARAGINE | ASN | N |
| ASPARTIC ACID | ASP | D |
| CYSTEINE | CYS | C |
| GLUTAMIC ACID | GLU | E |
| GLUTAMINE | GLN | Q |
| GLYCINE | GLY | G |
| HISTIDINE | HIS | H |
| ISOLEUCINE | ILE | I |
| LEUCINE | LEU | L |
| LYSINE | LYS | K |
| METHIONINE | MET | M |
| PHENYLALANINE | PHE | F |
| PROLINE | PRO | P |
| SERINE | SER | S |
| THREONINE | THR | T |
| TRYPTOPHAN | TRP | W |

| | | |
|----------|-----|---|
| TYROSINE | TYR | Y |
| VALINE | VAL | V |

PARTIAL AGONISTS shall mean moieties which activate the intracellular response when they bind to the receptor to a lesser degree/extent than do agonists, or enhance GTP binding to membranes to a lesser degree/extent than do agonists.

5 **ANTAGONIST** shall mean moieties that competitively bind to the receptor at the same site as the agonists but which do not activate the intracellular response initiated by the active form of the receptor, and can thereby inhibit the intracellular responses by agonists or partial agonists. **ANTAGONISTS** do not diminish the baseline intracellular response in the absence of an agonist or partial agonist.

10 **CANDIDATE COMPOUND** shall mean a molecule (for example, and not limitation, a chemical compound) which is amenable to a screening technique.

COMPOSITION shall mean a material comprising at least two compounds or two components; for example, and not limitation, a Pharmaceutical Composition is a Composition.

15 **COMPOUND EFFICACY** shall mean a measurement of the ability of a compound to inhibit or stimulate receptor functionality, as opposed to receptor binding affinity.

CONSTITUTIVELY ACTIVATED RECEPTOR shall mean a receptor subject to constitutive receptor activation.

20 **CONSTITUTIVE RECEPTOR ACTIVATION** shall mean stabilization of a receptor in the active state by means other than binding of the receptor with its endogenous ligand or a chemical equivalent thereof.

CONTACT or **CONTACTING** shall mean bringing at least two moieties together, whether in an in vitro system or an in vivo system.

25 **ENDOGENOUS** shall mean a material that a mammal naturally produces. **ENDOGENOUS** in reference to, for example and not limitation, the term "receptor" shall mean that which is naturally produced by a mammal (for example, and not limitation, a human) or a virus. In contrast, the term **NON-ENDOGENOUS** in this context shall mean that which is not naturally produced by a mammal (for example, and not limitation, a human) or a virus. For example, and not limitation, a receptor which is not constitutively active in its
30 endogenous form, but when manipulated becomes constitutively active, is most preferably

referred to herein as a “non-endogenous, constitutively activated receptor.” Both terms can be utilized to describe both “in vivo” and “in vitro” systems. For example, and not a limitation, in a screening approach, the endogenous or non-endogenous receptor may be in reference to an in vitro screening system. As a further example and not limitation, where the genome of a mammal has been manipulated to include a non-endogenous constitutively activated receptor, screening of a candidate compound by means of an in vivo system is viable.

INHIBIT or **INHIBITING**, in relationship to the term “response” shall mean that a response is decreased or prevented in the presence of a compound as opposed to in the absence of the compound.

INVERSE AGONISTS shall mean moieties that bind the endogenous form of the receptor or to the constitutively activated form of the receptor, and which inhibit the baseline intracellular response initiated by the active form of the receptor below the normal base level of activity which is observed in the absence of agonists or partial agonists, or decrease GTP binding to membranes. Preferably, the baseline intracellular response is inhibited in the presence of the inverse agonist by at least 30%, more preferably by at least 50%, and most preferably by at least 75%, as compared with the baseline response in the absence of the inverse agonist.

LIGAND shall mean an endogenous, naturally occurring molecule specific for an endogenous, naturally occurring receptor.

PHARMACEUTICAL COMPOSITION shall mean a composition comprising at least one active ingredient, whereby the composition is amenable to investigation for a specified, efficacious outcome in a mammal (for example, and not limitation, a human). Those of ordinary skill in the art will understand and appreciate the techniques appropriate for determining whether an active ingredient has a desired efficacious outcome based upon the needs of the artisan.

STIMULATE or **STIMULATING**, in relationship to the term “response” shall mean that a response is increased in the presence of a compound as opposed to in the absence of the compound.

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DETAILED DESCRIPTION

I. Particularly preferred mutations

For convenience, the sequence information regarding the non-endogenous, constitutively active human 5-HT_{2A} and 5-HT_{2C} receptors are referred to by identifiers as set forth in Table 2:

TABLE 2

| IDENTIFIER | RECEPTOR | SEQ.ID.NO: |
|------------|--------------------|------------|
| AP-1 cDNA | 5-HT _{2C} | 22 |
| AP-1 | 5-HT _{2C} | 23 |
| AP-3 cDNA | 5-HT _{2A} | 24 |
| AP-3 | 5-HT _{2A} | 25 |
| AP-4 cDNA | 5-HT _{2A} | 26 |
| AP-4 | 5-HT _{2A} | 27 |

As will be discussed in greater detail below, a mutation analogous to that reported by Casey (C322K) was utilized in the human 5-HT_{2A} receptor and is referred to herein as AP-2. However, AP-2 did not lead to sufficient constitutive activation to allow for utilization in screening techniques.

II. Generic G Protein-Coupled Receptor screening assay techniques

When a G protein receptor becomes constitutively active, it binds to a G protein (Gq, Gs, Gi, Go) and stimulates the binding of GTP to the G protein. The G protein then acts as a GTPase and slowly hydrolyzes the GTP to GDP, whereby the receptor, under normal conditions, becomes deactivated. However, constitutively activated receptors continue to exchange GDP to GTP. A non-hydrolyzable analog of GTP, [³⁵S]GTPγS, can be used to monitor enhanced binding to membranes which express constitutively activated receptors. It is reported that [³⁵S]GTPγS can be used to monitor G protein coupling to membranes in the absence and presence of ligand. An example of this monitoring, among other examples well-known and available to those in the art, was reported by Traynor and Nahorski in 1995. The preferred use of this assay system is for initial screening of candidate compounds because the

system is generically applicable to all G protein-coupled receptors regardless of the particular G protein that interacts with the intracellular domain of the receptor.

III. Confirmation of G Protein-Coupled Receptor site screening assay techniques

Once candidate compounds are identified using the “generic” G protein-coupled receptor assay (*i.e.* an assay to select compounds that are agonists, partial agonists, or inverse agonists), further screening to confirm that the compounds have interacted at the receptor site is preferred. For example, a compound identified by the “generic” assay may not bind to the receptor, but may instead merely “uncouple” the G protein from the intracellular domain. Thus, by further screening those candidate compounds, which have been identified using a “generic” assay in an agonist and/or antagonist competitive binding assay, further refinement in the selection process is provided.

Lysergic acid diethylamide (LSD) is a well-known agonist of the 5-HT_{2A} and 5-HT_{2C} receptors, while mesulergine is a well-known antagonist to the 5-HT_{2C} receptor. Accordingly, in most preferred embodiments, an agonist (LSD) and/or antagonist (mesulergine) competitive binding assay(s) is used to further screen those compounds selected from the “generic” assay for confirmation of serotonin receptor binding.

IV. Specified G Protein assay techniques

The art-accepted physiologically mediated pathway for the human 5-HT_{2A} and 5-HT_{2C} receptors is via Gq. Intracellular accumulation of IP3 can be used to confirm constitutive activation of these types of Gq coupled receptors (*see* Herrick-Davis-1). As a result, “IP3 accumulation” assays can be used to further screen those compounds selected from an agonist and/or antagonist competitive binding assay.

V. Pharmaceutical compositions

Candidate compounds selected for further development can be formulated into pharmaceutical compositions using techniques well known to those in the art. Suitable pharmaceutically-acceptable carriers are available to those in the art; for example, see Remington’s Pharmaceutical Sciences, 16th Edition, 1980, Mack Publishing Co., (Oslo et al., eds.)

EXAMPLES

The following examples are presented for purposes of elucidation, and not limitation, of the present invention. While specific nucleic acid and amino acid sequences are disclosed herein, those of ordinary skill in the art are credited with the ability to make minor modifications to these sequences while achieving the same or substantially similar

results reported below. It is intended that equivalent, non-endogenous, constitutively activated human serotonin receptor sequences include those having eighty-five percent (85%) homology, more preferably having ninety percent (90%) homology, and most preferably having ninety-five percent (95%) homology to the disclosed sequences.

5

EXAMPLE 1

GENERATION OF NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED HUMAN SEROTONIN RECEPTORS 5-HT_{2C} AND 5-HT_{2A}

A. Construction of constitutively active 5-HT_{2C} receptor cDNA

10

1. *Endogenous Human 5-HT_{2C}*

The cDNA encoding endogenous human 5-HT_{2C} receptor was obtained from human brain poly-A⁺ RNA by RT-PCR. The 5' and 3' primers were derived from the 5' and 3' untranslated regions and contained the following sequences:

5'-GACCTCGAGGTTGCTTAAGACTGAAGCA-3' (SEQ.ID.NO.:1)

15

5'-ATTTCTAGACATATGTAGCTTGTACCGT-3' (SEQ.ID.NO.:2)

PCR was performed using either TaqPlus™ precision polymerase (Stratagene) or rTth™ polymerase (Perkin Elmer) with the buffer systems provided by the manufacturers, 0.25 μM of each primer, and 0.2 mM of each of the four (4) nucleotides. The cycle condition was 30 cycles of 94°C for 1 minute, 57 °C for 1 minute and 72 °C for 2 minutes. The 1.5 kb PCR fragment was digested with Xho I and Xba I and subcloned into the Sal I-Xba I site of pBluescript.

20

The derived cDNA clones were fully sequenced and found to correspond to published sequences.

2. *AP-1 cDNA*

25

The cDNA containing a S310K mutation (AP-1 cDNA) in the third intracellular loop of the human 5-HT_{2C} receptor was constructed by replacing the Sty I restriction fragment containing amino acid 310 with synthetic double stranded oligonucleotides encoding the desired mutation. The sense strand sequence utilized had the following sequence:

5'-CTAGGGGCACCATGCAGGCTATCAACAATGAAAGAAAAGCTAAGAAAGTC-3'
(SEQ. ID.NO: 3)

30

and the antisense strand sequence utilized had the following sequence:

5'-CAAGGACTTTCTTAGCTTTTCTTTCATTGTTGATAGCCTGCATGGTGCCC-3'
(SEQ. ID. NO: 4).

B. Construction of constitutively active 5-HT_{2A} receptor cDNA**1. Endogenous Human 5-HT_{2A}**

The cDNA encoding endogenous human 5-HT_{2A} receptor was obtained by RT-PCR using human brain poly-A⁺ RNA; a 5' primer from the 5' untranslated region with a Xho I restriction site:

5'-GACCTCGAGTCCTTCTACACCTCATC-3' (SEQ.ID.NO:5)

and a 3' primer from the 3' untranslated region containing an Xba I site:

5'-TGCTCTAGATTCCAGATAGGTGAAAA CTTG-3' (SEQ.ID.NO:6).

PCR was performed using either TaqPlus™ precision polymerase (Stratagene) or rTth™ polymerase (Perkin Elmer) with the buffer systems provided by the manufacturers, 0.25 μM of each primer, and 0.2 mM of each of the four (4) nucleotides. The cycle condition was 30 cycles of 94°C for 1 minute, 57 °C for 1 minute, and 72 °C for 2 minutes. The 1.5 kb PCR fragment was digested with Xba I and subcloned into the Eco RV-Xba I site of pBluescript.

The resulting cDNA clones were fully sequenced and found to encode two amino acid changes from the published sequences. The first change is a T25N mutation in the N-terminal extracellular domain and the second change is an H452Y mutation. These mutations are likely to represent sequence polymorphisms rather than PCR errors since the cDNA clones having the same two mutations were derived from two independent PCR procedures using Taq polymerase from two different commercial sources (TaqPlus™ Stratagene and rTth™ Perkin Elmer).

2. Human 5-HT_{2A} (C322K; AP-2)

The cDNA containing the point mutation C322K in the third intracellular loop was constructed by using the Sph I restriction enzyme site, which encompasses amino acid 322. For the PCR procedure, a primer containing the C322K mutation:

5'-CAAAGAAAGTACTGGGCATCGTCTTCTTCCT-3' (SEQ.ID.NO:7)

was used along with the primer from the 3' untranslated region set forth above as SEQ.ID.NO:6. The resulting PCR fragment was then used to replace the 3' end of the wild type 5-HT_{2A} cDNA by the T4 polymerase blunted Sph I site. PCR was performed using pfu polymerase (Stratagene) with the buffer system provided by the manufacturer and 10% DMSO, 0.25 mM of each primer, 0.5mM of each of the 4 nucleotides. The cycle conditions were 25 cycles of 94°C for 1 minute, 60°C for 1 minute, and 72°C for 1 minute.

3. *AP-3 cDNA*

The human 5-HT_{2A} cDNA with intracellular loop 3 (IC3) or IC3 and cytoplasmic tail replaced by the corresponding human 5-HT_{2C} cDNA was constructed using PCR-based mutagenesis.

5 (a) *Replacement of IC3 Loop*

The IC3 loop of human 5-HT_{2A} cDNA was first replaced with the corresponding human 5-HT_{2C} cDNA. Two separate PCR procedures were performed to generate the two fragments, Fragment A and Fragment B, that fuse the 5-HT_{2C} IC3 loop to the transmembrane 6 (TM6) of 5-HT_{2A}. The 237 bp PCR fragment, Fragment A, containing
10 5-HT_{2C} IC3 and the initial 13 bp of 5-HT_{2A} TM6 was amplified by using the following primers:

5'-CCGCTCGAGTACTGCGCCGACAAGCTTTGAT-3' (SEQ.ID.NO:8)

5'-CGATGCCCAGCACTTTTGAAGCTTTTCTTTCATTGTTG-3' (SEQ.ID.NO:9)

The template used was human 5-HT_{2C} cDNA.

15 The 529 bp PCR fragment, Fragment B, containing the C-terminal 13 bp of IC3 from 5-HT_{2C} and the C-terminal of 5-HT_{2A} starting at beginning of TM6, was amplified by using the following primers:

5'-AAAAGCTTCGAAAGTGCTGGGCATCGTCTTCTTCCT-3' (SEQ.ID.NO:10)

5'-TGCTCTAGATTCCAGATAGGTGAAAACCTTG-3' (SEQ.ID.NO: 11)

20 The template used was human 5-HT_{2A} cDNA.

Second round PCR was performed using Fragment A and Fragment B as co-templates with SEQ.ID.NO:8 and SEQ.ID.NO:11 (it is noted that the sequences for SEQ.ID.NOS.: 6 and 11 are the same) as primers. The resulting 740 bp PCR fragment, Fragment C, contained the IC3 loop of human 5-HT_{2C} fused to TM6 through the end of the
25 cytoplasmic tail of human 5-HT_{2A}. PCR was performed using pfuTM polymerase (Stratagene) with the buffer system provided by the manufacturer, and 10% DMSO, 0.25 mM of each primer, and 0.5 mM of each of the four (4) nucleotides. The cycle conditions were 25 cycles of 94 °C for 1 minute, 57 °C (1st round PCR) or 60 °C (2nd round PCR) for 1 minute, and 72 °C for 1 minute (1st round PCR) or 90 seconds (2nd round PCR).

30 To generate a PCR fragment containing a fusion junction between the human 5-HT_{2A} TM5 and the IC3 loop of 5-HT_{2C}, four (4) primers were used. The two external primers, derived from human 5-HT_{2A}, had the following sequences:

5'-CGTGTCTCTCCTTACTTCA-3' (SEQ.ID.NO:12)

The other primer used was SEQ.ID.NO.:6 (*see* note above regarding SEQ.ID.NOS. 6 and 11). The first internal primer utilized was an antisense strand containing the initial 13 bp of IC3 of 5-HT_{2C} followed by the terminal 23 bp derived from TM5 of 5-HT_{2A}:

5'-TCGGCGCAGTACTTTGATAGTTAGAAAGTAGGTGAT-3' (SEQ.ID.NO:13)

5 The second internal primer was a sense strand containing the terminal 14 bp derived from TM5 of 5-HT_{2A} followed by the initial 24 bp derived from IC3 of 5-HT_{2C}:

5'-TTCTAACTATCAAAGTACTGCGCCGACAAGCTTTGATG-3' (SEQ.ID.NO:14).

PCR was performed using endogenous human 5-HT_{2A} and a co-template, Fragment C, in a 50 ml reaction volume containing 1X pfu buffer, 10% DMSO, 0.5 mM of each of the four (4) nucleotides, 0.25 mM of each external primer (SEQ.ID.NOS. 11 and 12), 0.06 mM of each internal primer (SEQ.ID.NOS. 13 and 14) and 1.9 units of pfu polymerase (Stratagene). The cycle conditions were 25 cycles of 94°C for 1 minute, 52°C for 1 minute, and 72 °C for 2 minutes and 10 seconds. The 1.3 kb PCR product was then gel purified and digested with Pst I and Eco RI. The resulting 1 kb PstI-Eco RI fragment
10 was used to replace the corresponding fragment in the endogenous human 5-HT_{2A} sequence to generate the mutant 5-HT_{2A} sequence encoding the IC3 loop of 5-HT_{2C}.
15

(b) Replacement of the cytoplasmic tail

To replace the cytoplasmic tail of 5-HT_{2A} with that of 5-HT_{2C}, PCR was performed using a sense primer containing the C-terminal 22 bp of TM7 of endogenous human 5-HT_{2A} followed by the initial 21 bp of the cytoplasmic tail of endogenous human 5-HT_{2C}:
20 5'-TTCAGCAGTCAACCCACTAGTCTATACTCTGTTCAACAAAATT-3'
(SEQ.ID.NO:15)

The antisense primer was derived from the 3' untranslated region of endogenous human 5-HT_{2C}:

25 5'-ATTTCTAGACATATGTAGCTTGTACCGT-3' (SEQ.ID.NO:16).

The resulting PCR fragment, Fragment D, contained the last 22 bp of endogenous human 5-HT_{2A} TM7 fused to the cytoplasmic tail of endogenous human 5-HT_{2C}. Second round PCR was performed using Fragment D and the co-template was endogenous human 5-HT_{2A} that was previously digested with Acc I to avoid undesired amplification. The
30 antisense primer used was SEQ.ID.NO:16 (the sequences for SEQ.ID.NOS. 16 and 2 are the same) and the sense primer used was derived from endogenous human 5-HT_{2A}:

5'-ATCACCTACTTTCTAACTA-3' (SEQ.ID.NO:17).

PCR conditions were as set forth in Example 1B3(a) for the first round PCR, except that the annealing temperature was 48 °C and the extension time was 90 seconds. The resulting 710 bp PCR product was digested with Apa I and Xba I and used to replace the corresponding Apa I-Xba I fragment of either (a) endogenous human 5-HT_{2A}, or (b) 5-HT_{2A} with 2C IC3 to generate (a) endogenous human 5-HT_{2A} with endogenous human 5-HT_{2C} cytoplasmic tail and (b) AP-3, respectively.

4. *AP-4 cDNA*

This mutant was created by replacement of the region of endogenous human 5-HT_{2A} from amino acid 247, the middle of TM5 right after Pro²⁴⁶, to amino acid 337, the middle of TM6 just before Pro³³⁸, by the corresponding region of AP-1 cDNA. For convenience, the junction in TM5 is referred to as the “2A-2C junction,” and the junction in TM6 is referred to as the “2C-2A junction.”

Three PCR fragments containing the desired hybrid junctions were generated. The 5' fragment of 561 bp containing the 2A-2C junction in TM5 was generated by PCR using endogenous human 5-HT_{2A} as template, SEQ.ID.NO:12 as the sense primer, and the antisense primer was derived from 13 bp of 5-HT_{2C} followed by 20 bp of 5-HT_{2A} sequence:

5'-CCATAATCGTCAGGGGAATGAAAAATGACACAA-3' (SEQ.ID.NO:18)

The middle fragment of the 323 bp contains endogenous human 5-HT_{2C} sequence derived from the middle of TM5 to the middle of TM6, flanked by 13 bp of 5-HT_{2A} sequences from the 2A-2C junction and the 2C-2A junction. This middle fragment was generated by using AP-1 cDNA as a template, a sense primer containing 13 bp of 5-HT_{2A} followed by 20 bp of 5-HT_{2C} sequences across the 2A-2C junction and having the sequence:

5'-ATTTTTCATTCCCCTGACGATTATGGTGATTAC-3' (SEQ.ID.NO:19);

and an antisense primer containing 13 bp of 5-HT_{2A} followed by 20 bp of 5-HT_{2C} sequences across the 2C-2A junction and having the sequence:

5'-TGATGAAGAAAGGGCACCACATGATCAGAAACA-3' (SEQ.ID.NO:20).

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The 3' fragment of 487 bp containing the 2C-2A junction was generated by PCR using endogenous human 5-HT_{2A} as a template and a sense primer having the following sequence from the 2C-2A junction:

5'-GATCATGTGGTGCCCTTTCTTCATCACAAACAT-3' (SEQ.ID.NO:21)

5 and the antisense primer was SEQ.ID.NO:6 (*see* note above regarding SEQ.ID.NOS. 6 and 11).

Two second round PCR reactions were performed separately to link the 5' and middle fragment (5'M PCR) and the middle and 3' fragment (M3' PCR). The 5'M PCR co-template used was the 5' and middle PCR fragment as described above, the sense primer was SEQ.ID.NO:12 and the antisense primer was SEQ.ID.NO:20. The 5'M PCR procedure resulted in an 857 bp PCR fragment.

The M3' PCR used the middle and M3' PCR fragment described above as the co-template, SEQ.ID.NO: 19 as the sense primer and SEQ.ID.NO:6 (*see* note above regarding SEQ.ID.NOS. 6 and 11) as the antisense primer, and generated a 784 bp amplification product. The final round of PCR was performed using the 857 bp and 784 bp fragments from the second round PCR as the co-template, and SEQ.ID.NO:12 and SEQ.ID.NO: 6 (*see* note above regarding SEQ.ID.NOS. 6 and 11) as the sense and the antisense primer, respectively. The 1.32 kb amplification product from the final round of PCR was digested with Pst I and Eco RI. Then resulting 1 kb Pst I-Eco RI fragment was used to replace the corresponding fragment of the endogenous human 5-HT_{2A} to generate mutant 5-HT_{2A} with 5-HT_{2C}: C310K/IC3. The Apa I-Xba fragment of AP3 was used to replace the corresponding fragment in mutant 5-HT_{2A} with 5-HT_{2C}: C310K/IC3 to generate AP4.

EXAMPLE 2

RECEPTOR EXPRESSION

A. pCMV

Although a variety of expression vectors are available to those in the art, for purposes of utilization for both the endogenous and non-endogenous receptors discussed herein, it is most preferred that the vector utilized be pCMV. This vector was deposited with the American Type Culture Collection (ATCC) on October 13, 1998 (10801 University Blvd., Manassas, VA 20110-2209 USA) under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure. The DNA was tested by the ATCC and determined to be viable. The

ATCC has assigned the following deposit number to pCMV: ATCC #203351. *See Figure 8.*

B. Transfection procedure

For the generic assay ($[^{35}\text{S}]\text{GTP}\gamma\text{S}$; Example 3) and the antagonist binding assay (mesulergine; Example 4), transfection of COS-7 or 293T cells was accomplished using the following protocol.

On day one, 5×10^6 COS-7 cells or 1×10^7 293T cells per 150mm plate were plated out. On day two, two reaction tubes were prepared (the proportions to follow for each tube are per plate): tube A was prepared by mixing 20 μg DNA (e.g., pCMV vector; pCMV vector AP-1 cDNA, etc.) in 1.2ml serum free DMEM (Irvine Scientific, Irvine, CA); tube B was prepared by mixing 120 μl lipofectamine (Gibco BRL) in 1.2ml serum free DMEM. Tubes A and B were then admixed by inversions (several times), followed by incubation at room temperature for 30-45min. The admixture is referred to as the "transfection mixture". Plated COS-7 cells were washed with 1X PBS, followed by addition of 10ml serum free DMEM. 2.4ml of the transfection mixture was then added to the cells, followed by incubation for 4hrs at 37°C/5% CO_2 . The transfection mixture was then removed by aspiration, followed by the addition of 25ml of DMEM/10% Fetal Bovine Serum. Cells were then incubated at 37°C/5% CO_2 . After 72hr incubation, cells were then harvested and utilized for analysis.

EXAMPLE 3

PROTOCOL:

GTP MEMBRANE BINDING SCINTILLATION PROXIMITY ASSAY

The advantages of using $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding to measure constitutive activation are that: (a) $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding is generically applicable to all G protein-coupled receptors; and (b) $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding is proximal at the membrane surface, thereby making it less likely to pick-up molecules which affect the intracellular cascade. The assay utilizes the ability of G protein-coupled receptors to stimulate $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ binding to membranes expressing the relevant receptors. Therefore, the assay may be used to directly screen compounds at the disclosed serotonin receptors.

A scintillation proximity assay can be utilized to monitor the binding of $[^{35}\text{S}]\text{GTP}\gamma\text{S}$ to membranes expressing, e.g., the endogenous human 5-HT_{2C} receptor expressed in COS cells. In brief, a preferred protocol for the assay is such that the assay was incubated in 20

mM HEPES, pH 7.4, binding buffer with 0.3 nM [35 S]GTP γ S and 12.5 μ g membrane protein and 1 μ M GDP for 30 minutes. Wheatgerm agglutinin beads (25 μ l; Amersham) were then added and the mixture was incubated for another 30 minutes at room temperature. The tubes were then centrifuged at 1500 x g for 5 minutes at room temperature and then counted in a scintillation counter. Serotonin, which as the endogenous ligand activates the 5-HT $_2$ C receptor, stimulated [35 S]GTP γ S binding to the membranes in a concentration dependant manner (data not shown). The stimulated binding can be completely inhibited by 30 μ M mianserin, a compound considered as a classical 5-HT $_2$ C antagonist, but also known as a 5-HT $_2$ C inverse agonist (data not shown).

Although this assay measures agonist-induced binding of [35 S]GTP γ S to membranes and can be routinely used to measure constitutive activity of receptors, the present cost of wheatgerm agglutinin beads may be prohibitive. A less costly but equally applicable alternative also meets the needs of large-scale screening. Flash plates and WallacTM scintistrips may be used to format a high throughput [35 S]GTP γ S binding assay. This technique allows one to monitor the tritiated ligand binding to the receptor while simultaneously monitoring the efficacy via [35 S]GTP γ S binding. This is possible because the WallacTM beta counter can switch energy windows to analyze both tritium and 35 S-labeled probes.

Also, this assay may be used for detecting of other types of membrane activation events that result in receptor activation. For example, the assay may be used to monitor 32 P phosphorylation of a variety of receptors (including G protein-coupled and tyrosine kinase receptors). When the membranes are centrifuged to the bottom of the well, the bound [35 S]GTP γ S or the 32 P-phosphorylated receptor will activate the scintillant coated on the wells. Use of Scinti[®] strips (WallacTM) demonstrate this principle. Additionally, this assay may be used for measuring ligand binding to receptors using radiolabeled ligands. In a similar manner, the radiolabeled bound ligand is centrifuged to the bottom of the well and activates the scintillant. The [35 S]GTP γ S assay results parallel the results obtained in traditional second messenger assays of receptors.

In this assay, serotonin stimulates the binding of [35 S]GTP γ S to the endogenous human 5-HT $_2$ C receptor, while mianserin inhibits this response; furthermore, mianserin acts as a partial inverse agonist by inhibiting the basal constitutive binding of [35 S]GTP γ S to membranes expressing the endogenous human 5-HT $_2$ C receptor (data not shown). In this

assay, there should be no agonist response in the absence of GDP since there is no GDP present to exchange for [³⁵S]GTPγS. This assay system can be used to demonstrate the response of the native 5-HT_{2C} receptor, and to also measures the constitutive activation of other receptors.

5 Using this assay, enhanced binding of [³⁵S]GTPγS to membranes prepared from 293T cells expressing the control vector alone, the native human 5-HT_{2C} receptor or the AP-1 receptor was observed (data not shown). The total protein concentration used in the assay affects the total amount of [³⁵S]GTPγS binding for each receptor. The c.p.m. differential between the pCMV transfected and the constitutively active mutant receptor increased from
10 approximately 1000 c.p.m at 10 μg/well to approximately 6-8000 c.p.m. at 75 μg/well protein concentration.

The AP-1 receptor showed the highest level of constitutive activation followed by the wild type receptor, which also showed enhanced [³⁵S]GTPγS binding above basal. This is consistent with the ability of the endogenous human 5-HT_{2C} receptor to accumulate
15 intracellular IP3 in the absence of 5HT stimulation (Example 5) and is also consistent with published data claiming that the endogenous human 5-HT_{2C} receptor has a high natural basal activity. Therefore, the AP-1 receptor demonstrates that constitutive activity may be measured by proximal [³⁵S]GTPγS binding events at the membrane interface.

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EXAMPLE 4

PROTOCOL:

SEROTONIN RECEPTOR AGONIST/ANTAGONIST COMPETITIVE BINDING ASSAY

25 Membranes were prepared from transfected COS-7 cells (*see* Example 2) by homogenization in 20 mM HEPES and 10 mM EDTA, pH 7.4 and centrifuged at 49,000 x g for 15 min. The pellet was resuspended in 20 mM HEPES and 0.1 mM EDTA, pH 7.4, homogenized for 10 sec. using polytron homogenizer (Brinkman) at 5000 rpm and centrifuged at 49,000 x g for 15 min. The final pellet was resuspended in 20 mM HEPES
30 and 10 mM MgCl₂, pH 7.4, homogenized for 10 sec. using polytron homogenizer (Brinkman) at 5000 rpm.

Assays were performed in triplicate 200μl volumes in 96 well plates. Assay buffer (20 mM HEPES and 10 mM MgCl₂, pH 7.4) was used to dilute membranes, ³H-LSD, ³H-mesulergine, serotonin (used to define non-specific for LSD binding) and mianserin (used

to define non-specific for mesulergine binding). Final assay concentrations consisted of 1nM ^3H -LSD or 1nM ^3H -mesulergine, 50 μg membrane protein and 100 μM serotonin or mianserin. LSD assays were incubated for 1 hr at 37° C, while mesulergine assays were incubated for 1 hr at room temperature. Assays were terminated by rapid filtration onto
5 Wallac Filtermat Type B with ice cold binding buffer using Skatron cell harvester. The radioactivity was determined in a Wallac 1205 BetaPlate counter.

EXAMPLE 5

PROTOCOL:

INTRACELLULAR IP_3 ACCUMULATION ASSAY

For the IP_3 accumulation assay, a transfection protocol different from the protocol set forth in Example 2 was utilized. In the following example, the protocols used for days 1-3 were slightly different for indicated conditions, as set forth below; the protocol for day 4 was the same for all conditions.

A. COS-7 and 293 Cells

On day one, COS-7 cells or 293 cells were plated onto 24 well plates, usually 1x10⁵ cells/well or 2x10⁵ cells/well, respectively. On day two, the cells were transfected by first mixing 0.25 μg DNA (*see* Example 2) in 50 μl serum-free DMEM/well and then 2 μl lipofectamine in 50 μl serum-free DMEM/well. The solutions ("transfection media")
20 were gently mixed and incubated for 15-30 minutes at room temperature. The cells were washed with 0.5 ml PBS and then 400 μl of serum free media was mixed with the transfection media and added to the cells. The cells were then incubated for 3-4 hours at 37°C/5%CO₂. Then the transfection media was removed and replaced with 1ml/well of regular growth media. On day 3, the media was removed and the cells were washed with
25 0.5 ml PBS. Then 0.5 ml inositol-free/serum-free media (GIBCO BRL) was added to each well with 0.25 μCi of ^3H -myo-inositol/well and the cells were incubated for 16-18 hours overnight at 37°C/5%CO₂. Protocol A.

B. 293 Cells

On day one, 1x10⁷ 293 cells per 150mm plate were plated out. On day two, two
30 reaction tubes were prepared (the proportions to follow for each tube are per plate): tube A was prepared by mixing 20 μg DNA (e.g., pCMV vector; pCMV vector AP-1 cDNA, etc.) in 1.2ml serum free DMEM (Irvine Scientific, Irvine, CA); tube B was prepared by mixing 120 μl lipofectamine (Gibco BRL) in 1.2ml serum free DMEM. Tubes A and B were then

admixed by inversions (several times), followed by incubation at room temperature for 30-45min. The admixture is referred to as the "transfection mixture". Plated 293 cells were washed with 1XPBS, followed by addition of 10ml serum free DMEM. 2.4ml of the transfection mixture was then added to the cells, followed by incubation for 4hrs at 37°C/5% CO₂. On day 3, cells were trypsinized and counted, followed by plating of 1x10⁶ cells/well (poly D-lysine treated 12-well plates). Cells were permitted to adhere to the wells, followed by one wash with 1xPBS. Thereafter, 0.5 µCi ³H-inositol in 1ml inositol-free DMEM was added per well. Protocol B.

On day 4, the cells were washed with 0.5 ml PBS and then 0.45 ml of assay medium was added containing inositol-free/serum free media, 10 µM pargyline, 10 mM lithium chloride, or 0.4 ml of assay medium and 50 µl of 10x ketanserin (ket) to a final concentration of 10µM. The cells were then incubated for 30 minutes at 37°C. Then the cells were washed with 0.5 ml PBS and 200 µl of fresh/ice cold stop solution (1M KOH; 18 mM Na-borate; 3.8 mM EDTA) was added/well. The solution was kept on ice for 5-10 minutes or until the cells were lysed and then neutralized by 200 µl of fresh/ice cold neutralization sol. (7.5 % HCL). The lysate was then transferred into 1.5 ml micro-centrifuge tubes and 1 ml of chloroform/methanol (1:2) was added/tube. The solution was vortexed for 15 seconds and the upper phase was applied to a Biorad AG1-X8 anion exchange resin (100-200 mesh). The resin was washed with water and 0.9 ml of the upper phase was loaded onto the column. The column was washed with 10 mls of 5 mM myo-inositol and 10 ml of 5 mM Na-borate/60mM Na-formate. The inositol trisphosphates were eluted into scintillation vials containing 10 ml of scintillation cocktail with 2 ml of 0.1 M formic acid/ 1 M ammonium formate. The columns were regenerated by washing with 10 ml of 0.1 M formic acid/3M ammonium formate and rinsed twice with dd H₂O and stored at room temperature in water. Results are discussed below.

EXAMPLE 7

SCREENING OF CANDIDATE COMPOUNDS AGAINST NON-ENDOGENOUS, CONSTITUTIVELY ACTIVATED HUMAN SEROTONIN RECEPTORS: AP-1

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Approximately 5,500 candidate compounds (Tripos, Inc., St. Louis, MO) were screened using the assay protocol of Example 3 (with AP-1 mutant receptor) for identification as inverse agonists against the receptor; for this assay, an arbitrary cut-off of at least 50%

inhibition was established for identification of inverse agonists. Approximately 120 of these compounds evidenced at least 50% inhibition of [³⁵S]GTP γ S binding at 10 μ M candidate compound (data not shown).

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EXAMPLE 8**SCREENING OF SELECTED COMPOUNDS TO CONFIRM RECEPTOR BINDING: AP-1**

The candidate compounds identified from Example 7 were then screened using the assay protocol of Example 4 (mesulergine), using the AP-1 mutant receptor. IC₅₀ (nM) values were determined; five of the nearly 120 compounds of Example 7 were determined to have potent binding affinity for the receptor. Results are summarized in Table 4.

Table 4

| Candidate Compound | IC ₅₀ (nM) in Mesulergine Assay |
|--------------------|--|
| 102461 | 205.0 |
| 102788 | 46.5 |
| 100341 | 209.0 |
| 100431 | 147.0 |
| 103487 | 1,810.0 |

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EXAMPLE 9a**GENERAL SCREENING PARADIGM:
SELECTION OF PRE-CLINICAL CANDIDATE LEADS**

The “primary” screen designed to directly identify human 5HT_{2A}/5HT_{2C} receptor inverse agonists consisted of a membrane-based GTP γ S binding assay utilizing membranes prepared from COS7 cells transiently transfected with AP-1 human receptor. Candidate compounds (10 μ M final assay concentration) directly identified as inhibiting receptor-mediated increases in GTP γ S binding by greater than 50-75% (arbitrary cut-off value) were considered active “hits”. Primary assay hits were then re-tested in the same assay to reconfirm their inverse agonist activity. If primary assay hits were reconfirmed active (50% or greater inhibition), and therefore directly identified as, *e.g.*, an inverse

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agonist, one of two approaches were available: (a) so-called "directed libraries" could be created, *i.e.*, additional candidate compounds were synthesized based upon the structures of the reconfirmed hits (geared towards, *e.g.*, improvement in the characteristics of the compounds) whereby the directed library compounds were then evaluated for the ability to compete for radioligand binding to both mutant 5HT_{2C} (AP-1) and endogenous 5HT_{2A} receptors, or (b) the reconfirmed hits were then evaluated for the ability to compete for radioligand binding to both mutant 5HT_{2C} (AP-1) and endogenous 5HT_{2A} receptors. Thus, when approach (a) was used, because these directed library candidate compounds were based upon the structures of compounds that were directly identified from the membrane-based GTPγS binding assay, the directed library compounds were not re-tested in the membrane-based GTPγS binding assay but rather were then confirmed via the radioligand binding analysis. The radioligand binding analysis tests were initially performed at 10μM test compound in triplicate and if the compound inhibited radiolabeled binding by 50% or more, the analysis was followed by eight concentration competition curves to determine Ki values. The last step in secondary assay evaluation was to determine if test compounds were capable of inhibiting AP-3 receptor-mediated accumulation of inositol phosphates (*e.g.*, IP₃). This final assay confirms that the directly identified compounds retained inverse agonist properties.

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EXAMPLE 9b

CONSTITUTIVELY ACTIVATED HUMAN 5HT_{2C} RECEPTOR (AP-1) MEDIATED FACILITATION OF GTPγS BINDING TO COS7 MEMBRANES

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This protocol is substantially the same as set forth above in Example 6.

Primary screening assays measuring GTPγS binding to membranes prepared from COS7 cells transiently transfected with human mutated 5HT_{2C} receptor (AP-1) were used to directly identify inverse agonists in screening libraries (Tripos, Inc.). Candidate compound screens were performed in a total assay volume of 200μl using scintillant-coated Wallac Scintistrip™ plates. The primary assay was comprised of the following chemicals (at indicated final assay concentrations): 20 mM HEPES, pH 7.4, 100 mM NaCl, 20 mM MgCl₂, 0.2% saponin, 0.2 mM ascorbic acid, 1μM GDP, 0.3 nM GTPγ³⁵S, and 12.5 μg of the above defined membranes. Incubations were performed for 60 minutes at ambient room temperature. The binding assay incubation was terminated by

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centrifugation of assay plates at 4,000 rpm for 15 minutes, followed by rapid aspiration of the reaction mixture and counting in a Wallac MicroBeta™ scintillation counter.

Primary screening of candidate compounds initially involved testing of 72 test compounds per assay plate (96-well plates were utilized), at a final assay concentration of 10μM candidate compound, in single replicates. A total of sixteen wells of each plate were dedicated for an eight concentration clozapine (a confirmed 5HT_{2C/2A} inverse agonist) dose response curve (duplicate determinations at each concentration). Finally, a total of five assay wells of each plate were dedicated to define the negative control (AP-1 receptor expressing membranes without addition of candidate compounds) and three wells from each plate to define the positive control (membranes without AP-1 receptor).

Reconfirmation experiments involve re-testing candidate compounds in the same assay described above, except that candidate compounds were evaluated in triplicate, thus allowing evaluation of 24 compounds per 96-well assay plate. Similar to the primary assay plates, an eight concentration clozapine dose response curve (duplicate determinations at each concentration) and the same negative and positive control wells were also included within each 96-well plate.

EXAMPLE 9c(1)

COMPETITION STUDIES FOR DIRECTLY IDENTIFIED COMPOUNDS: MUTATED HUMAN 5HT_{2C} RECEPTOR (AP-1)

Radioligand binding competition experiments were performed in a total assay volume of 200μl using standard 96-well microtiter plates. The final assay ingredients consisted of assay buffer (20mM HEPES and 10mM MgCl₂), 1nM [³H]mesulergine, and 50μg of membranes (COS7 with AP-1 as defined above). Nonspecific [³H]mesulergine binding was defined in the presence of 100μM mianserin. Incubations were performed for 1 hour at 37°C. Receptor bound radioligand was resolved from free radioligand by rapid filtration of the assay mixture over a Wallac Filtermat™ Type B filter, followed by washing with ice-cold assay buffer using a Skatron™ cell harvester. Radioactivity was counted using a Wallac 1205 BetaPlate™ counter. Each assay plate contained five negative control wells (membranes expressing receptor and no candidate compound addition) and three positive control wells (each containing 100μM mianserin). For one concentration tests, candidate compounds were diluted into assay buffer and screened at a final concentration of 10μM, in

triplicate. For IC₅₀ determinations, candidate compounds were diluted in assay buffer and eight different concentrations were evaluated, in triplicate. A total of 16 wells were designated for an eight concentration mianserin dose response curve evaluation for both assays.

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EXAMPLE 9c(2)

COMPETITION STUDIES WILD TYPE HUMAN 5HT_{2A} RECEPTOR

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Radioligand binding competition experiments were performed in a total assay volume of 200µl using standard 96-well microtiter plates. The final assay ingredients comprised assay buffer (20mM HEPES and 10mM MgCl₂), 1nM [³H]LSD, and 50µg of the above-defined membranes (COS7 with AP-1). Nonspecific [³H]LSD binding was defined in the presence of 100µM serotonin. Incubations were performed for 1 hour at 37°C. Receptor bound radioligand was resolved from free radioligand by rapid filtration of the assay mixture over a Wallac Filtermat™ Type B filter, followed by washing with ice-cold assay buffer using a Skatron™ cell harvester. Radioactivity was counted using a Wallac 1205 BetaPlate™ counter. Each assay plate contained five negative control wells (membranes expressing receptor and no candidate compound addition) and three positive control wells (containing 100µM mianserin). For one concentration tests, candidate compounds were diluted into assay buffer and screened at a final concentration of 10µM in triplicate. For IC₅₀ determinations, candidate compounds were diluted in assay buffer and eight different concentrations were evaluated in triplicate. A total of 16 wells were designated for an eight concentration serotonin dose response curve evaluation for both assays.

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EXAMPLE 9d

RECEPTOR-MEDIATED INOSITOL PHOSPHATE ACCUMULATION

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Candidate compound identified in the assays of Examples 9a-9c were then evaluated for inositol phosphate accumulation, following the protocol of Example 5 (COS7 cells expressing human mutated 5HT_{2A} receptor, AP-3), modified as follows: tube A was prepared by mixing 16µg DNA (e.g., pCMV vector; pCMV vector AP-1 cDNA, etc.) in 1.0ml serum free DMEM (Irvine Scientific, Irvine, CA); tube B was prepared by mixing

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60µl lipofectamine (Gibco BRL) in 1.0ml serum free DMEM. Tubes A and B were then admixed by inversions (several times), followed by incubation at room temperature for 30min. The admixture is referred to as the "transfection mixture". Plated 293 cells were washed with 10ml Serum Free DMEM, followed by addition of 11ml Serum Free DMEM. 2.0ml of the transfection mixture was then added to the cells, followed by incubation for 5hrs at 37°C/5% CO₂. On day 3, cells were trypsinized and counted, followed by plating of 1x10⁶ cells/well (12-well plates). Cells were permitted to adhere to the wells for 8hrs, followed by one wash with 1xPBS. Thereafter, 0.5 µCi ³H-inositol in 1ml inositol-free DMEM was added per well.

On day 4, the cells were washed with 1.5 ml PBS and then 0.9 ml of assay medium was added containing inositol-free/serum free media, 10 µM pargyline, 10 mM lithium chloride, for 5min in 37°C/5% CO₂ followed by 100µl addition of candidate compound diluted in the same material. The cells were then incubated for 120 minutes at 37°C. Then the cells were washed with 1.5 ml PBS and 200 µl of fresh/icecold stop solution (1M KOH; 18 mM Na-borate; 3.8 mM EDTA) was added/well. The solution was kept on ice for 5-10 minutes or until the cells were lysed and then neutralized by 200 µl of fresh/ice cold neutralization sol. (7.5 % HCL). The lysate was then transferred into 1.5 ml micro-centrifuge tubes and 1 ml of chloroform/methanol (1:2) was added/tube. The solution was vortexed for 15 seconds and the upper phase was applied to a Biorad AG1-X8 anion exchange resin (100-200 mesh). The resin was washed with water and 0.9 ml of the upper phase was loaded onto the column. The column was washed with 10 mls of 5 mM myo-inositol and 10 ml of 5 mM Na-borate/60mM Na-formate. The inositol trisphosphates were eluted into scintillation vials containing 10 ml of scintillation cocktail with 2 ml of 0.1 M formic acid/ 1 M ammonium formate. The columns were regenerated by washing with 10 ml of 0.1 M formic acid/3M ammonium formate and rinsed twice with dd H₂O and stored at room temperature in water.

Following this round of assaying, candidate compounds having an IC₅₀ value of less than 10µM were considered as potential leads for the development of pharmaceutical compositions.

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EXAMPLE 10**SCREENING CANDIDATE COMPOUNDS**

Following the protocols set forth above, one compound, 103487 (Example 8, *supra*) evidenced the following results:

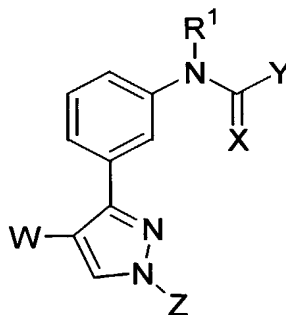
5

| Compound No. | GTP γ S AP-1 Percent Inhibition Relative To Positive Control (Primary) | GTP γ S AP-1 Percent Inhibition Relative To Positive Control (Reconfirm) | Competitive Binding AP-1 ([3 H]mesulergine) IC ₅₀ Value (nM) | Competitive Binding WT 5HT _{2A} ([3 H]LSD) IC ₅₀ Value (nM) | Inositol Phosphate Accumulation AP-3 IC ₅₀ Value (nM) |
|--------------|---|---|--|--|---|
| 103487 | -1% | 31% | 2100 850 | 46 | 52 90 |

Based upon these results, structure activity analysis of the 103487 compound suggested that a series of derivatives of 3-(4-bromo-1-methylpyrazole-3-yl)phenylamine would exhibit similar 5-HT_{2A} activity and selectivity. A series of derivatives of 3-(4-bromo-1-methylpyrazole-3-yl)phenylamine were synthesized. These “directed” library compounds (Tripos, Inc.) were then analyzed in accordance with the protocols of Examples 9c(1), 9c(2) and 9d.

A preferred series of compounds possessing 5-HT_{2A} receptor activity that are useful as inverse agonists at such receptors is designated by the general Formula (A):

15



(A)

wherein:

W is F, Cl, Br, I, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl;

X is O or S or NR²;

Y is NR³R⁴, or (CH₂)_mR⁵, or O(CH₂)_nR⁶

5 **m** is an integer between 0 and 4, inclusive;

n is an integer between 0 and 4, inclusive;

Z is H, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl;

10 **R**¹, **R**², **R**³ and **R**¹⁰ are each independently selected from: H, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl;

R⁴, **R**⁵, and **R**⁶ is each independently selected from: C₁₋₈ straight chain or branched alkyl, C₂₋₈ alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl, or CH₂aryl, wherein each moiety within said C₁₋₈ straight chain or branched alkyl, C₂₋₈ alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH₂aryl may be optionally substituted by up to four substituents
15 in any position, whereby each substituent is independently selected from: H, F, Cl, Br, I, R⁷, CF₃, CF₂R⁷, CF₂CF₂, CCl₃, CCl₂R⁷, CCl₂CCl₂R⁷, NR⁸R⁹, NR¹⁰COR⁷, NR¹⁰SO₂R⁷, OR⁷, OCF₃, OCF₂R⁷, OCF₂CF₂R⁷, OCOR⁷, OSO₂R⁷, OPO(OR⁷)₂, SR⁷, SCF₃, SCF₂R⁷, SCF₂CF₂R⁷, SCOR⁷, SO₃R⁷, SO₂NR⁸R⁹, PO(OR⁷)₃, PO(OR⁷)₂R⁷, NO₂, CN, CNR¹⁰(NR⁸R⁹), CNR¹⁰(SR⁷), COOR⁷, COSR⁷, CONR⁸R⁹,

20 with the proviso that when **R**⁴, **R**⁵, or **R**⁶ contains an aryl ring substituted at at least two adjacent positions on said aryl ring, then said two adjacent positions can together be selected from SCH₂S, SCH₂CH₂S, OCH₂O, or OCH₂CH₂O to form a bi-cyclic structure;

25 **R**⁷ is H, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, C₂₋₈ alkenyl, aryl or alkylaryl; and

R⁸ and **R**⁹ are each independently selected from H, C₁₋₈ straight chain or branched alkyl, C₂₋₈ alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl, or CH₂aryl, wherein each moiety within said C₁₋₈ straight chain or branched alkyl, C₂₋₈ alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH₂aryl may be optionally substituted by up to four substituents
30 in any position, whereby each substituent is independently selected from: F, Cl, Br, I, CF₃, CCl₃, CH₃, C₂H₅, C₃H₇, C₄H₉, NH₂, NHCH₃, N(CH₃)₂, NHC₂H₅, N(C₂H₅)₂, NHC₃H₇, N(C₃H₇)₂, NHC₄H₉, N(C₄H₉)₂, NHCOH, NHCOCH₃, NHCOC₂H₅, NHCOC₃H₇, NHCOC₄H₉, NHSO₂CH₃, NHSO₂C₂H₅, NHSO₂C₃H₇, NHSO₂C₄H₉, OH, OCH₃, OC₂H₅,

OC₃H₇, OC₄H₇, OC₄H₉, OC₅H₉, OC₅H₁₁, OC₆H₁₁, OC₆H₁₃, OCF₃, OCOCH₃, OCOC₂H₅,
 OCOC₃H₇, OCOC₄H₉, OSO₂CH₃, OSO₂C₂H₅, OSO₂C₃H₇, OSO₂C₄H₉, SH, SCH₃, SC₂H₅,
 SC₃H₇, SC₄H₇, SC₄H₉, SC₅H₉, SC₅H₁₁, SC₆H₁₁, SC₆H₁₃, SCF₃, SCOCH₃, SCOC₂H₅,
 SCOC₃H₇, SCOC₄H₉, SO₃CH₃, SO₃C₂H₅, SO₃C₃H₇, SO₃C₄H₉, SO₂NH, SO₂NH₂,
 5 SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅, SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂,
 SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN, COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉,
 COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉, CONH₂, CONHCH₃, CON(CH₃)₂,
 CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂, CONHC₄H₉, CON(C₄H₉)₂, and

with the proviso that when either or both of **R**⁸ or **R**⁹ contain an aryl ring
 10 substituted at at least two adjacent positions on said aryl ring, then said two
 adjacent positions can together be selected from SCH₂S, SCH₂CH₂S,
 OCH₂O, or OCH₂CH₂O to form a bi-cyclic structure;

or

R⁸ and **R**⁹ may together form part of a 5, 6 or 7 membered cyclic structure, with
 15 said structure being saturated or unsaturated, and further with said structure containing up
 to four heteroatoms selected from O, N or S, and further wherein each moiety within said
 cyclic structure being optionally substituted by up to four substituents in any position
 independently selected from: F, Cl, Br, I, CF₃, CCl₃, CH₃, C₂H₅, C₃H₇, C₄H₉, NH₂,
 NHCH₃, N(CH₃)₂, NHC₂H₅, N(C₂H₅)₂, NHC₃H₇, N(C₃H₇)₂, NHC₄H₉, N(C₄H₉)₂, NHCOH,
 20 NHCOCH₃, NHCOC₂H₅, NHCOC₃H₇, NHCOC₄H₉, NHSO₂CH₃, NHSO₂C₂H₅,
 NHSO₂C₃H₇, NHSO₂C₄H₉, OH, OCH₃, OC₂H₅, OC₃H₇, OC₄H₇, OC₄H₉, OC₅H₉, OC₅H₁₁,
 OC₆H₁₁, OC₆H₁₃, OCF₃, OCOCH₃, OCOC₂H₅, OCOC₃H₇, OCOC₄H₉, OSO₂CH₃,
 OSO₂C₂H₅, OSO₂C₃H₇, OSO₂C₄H₉, SH, SCH₃, SC₂H₅, SC₃H₇, SC₄H₇, SC₄H₉, SC₅H₉,
 SC₅H₁₁, SC₆H₁₁, SC₆H₁₃, SCF₃, SCOCH₃, SCOC₂H₅, SCOC₃H₇, SCOC₄H₉, SO₃CH₃,
 25 SO₃C₂H₅, SO₃C₃H₇, SO₃C₄H₉, SO₂NH, SO₂NH₂, SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅,
 SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂, SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN,
 COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉, COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉,
 CONH₂, CONHCH₃, CON(CH₃)₂, CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂,
 CONHC₄H₉, CON(C₄H₉)₂, and

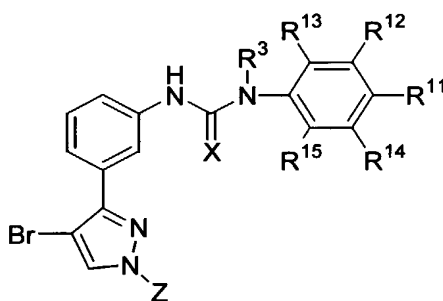
30 with the proviso that wherein when **R**⁸ and **R**⁹ form an aryl ring substituted
 at at least two adjacent positions on said aryl ring, then said two adjacent
 positions can together be selected from SCH₂S, SCH₂CH₂S, OCH₂O, or
 OCH₂CH₂O to form a bi-cyclic structure.

An **aryl moiety** can be a 5 or 6 membered aromatic heterocyclic ring (containing up to 4 hetero atoms independently selected from N, O, or S) or a 6 membered aromatic non-heterocyclic ring or a polycycle;

5 Examples of suitable **C₁₋₈ alkyl groups** include but are not limited to methyl, ethyl, n-propyl, i-propyl, n-butyl, and t-butyl.

 Examples of **5 or 6 membered ring moieties** include, but are not restricted to, phenyl, furanyl, thienyl, imidazolyl, pyridyl, pyrrolyl, oxazolyl, isoxazolyl, triazolyl, pyrazolyl, tetrazolyl, thiazolyl, and isothiazolyl. Examples of polycycle moieties include,
10 but are not restricted to, naphthyl, benzothiazolyl, benzofuranyl, benzimidazolyl, quinolyl, isoquinolyl, indolyl, quinoxaliny, quinazolinyl, and benzothienyl.

 Preferred compounds falling within the scope of general Formula (A) as Class (1) compounds where $Y = NR^3R^4$ are as follows:



(1)

wherein:

X is O or S or NR^2 ;

Z is H or CH_3 ;

R², R³ and R¹⁰ are each independently selected from H, C₁₋₈ straight chain or
20 branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl;

R¹¹ is H, F, Cl, Br, I, R^7 , CF_3 , CF_2R^7 , CF_2CF_2 , CCl_3 , CCl_2R^7 , $CCl_2CCl_2R^7$, NR^8R^9 , $NR^{10}COR^7$, $NR^{10}SO_2R^7$, OR^7 , OCF_3 , OCF_2R^7 , $OCF_2CF_2R^7$, $OCOR^7$, OSO_2R^7 , $OPO(OR^7)_2$, SR^7 , SCF_3 , SCF_2R^7 , $SCF_2CF_2R^7$, $SCOR^7$, SO_3R^7 , $SO_2NR^8R^9$, $PO(OR^7)_3$, $PO(OR^7)_2R^7$, NO_2 , CN , $CNR^{10}(NR^8R^9)$, $CNR^{10}(SR^7)$, $COOR^7$, $COSR^7$, $CONR^8R^9$,

with the proviso that when a position adjacent to R^{11} is substituted, then R^{11} and said adjacent position can together be selected from SCH_2S , SCH_2CH_2S , OCH_2O , or OCH_2CH_2O to form a bi-cyclic structure;

R^7 is H, C_{1-8} straight chain or branched alkyl, C_{3-8} cycloalkyl, C_{4-9} alkylcycloalkyl,
5 C_{2-8} alkenyl, aryl or alkylaryl;

R^8 and R^9 are each independently selected from: H, C_{1-8} straight chain or branched alkyl, C_{2-8} alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH_2 aryl wherein each moiety within said C_{1-8} straight chain or branched alkyl, C_{2-8} alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH_2 aryl may be optionally substituted by up to four substituents
10 in any position, whereby each substituent is independently selected from: F, Cl, Br, I, CF_3 , CCl_3 , CH_3 , C_2H_5 , C_3H_7 , C_4H_9 , NH_2 , $NHCH_3$, $N(CH_3)_2$, NHC_2H_5 , $N(C_2H_5)_2$, NHC_3H_7 , $N(C_3H_7)_2$, NHC_4H_9 , $N(C_4H_9)_2$, $NHCOH$, $NHCOCH_3$, $NHCOC_2H_5$, $NHCOC_3H_7$, $NHCOC_4H_9$, $NHSO_2CH_3$, $NHSO_2C_2H_5$, $NHSO_2C_3H_7$, $NHSO_2C_4H_9$, OH , OCH_3 , OC_2H_5 , OC_3H_7 , OC_4H_7 , OC_4H_9 , OC_5H_9 , OC_5H_{11} , OC_6H_{11} , OC_6H_{13} , OCF_3 , $OCOCH_3$, $OCOC_2H_5$,
15 $OCOC_3H_7$, $OCOC_4H_9$, OSO_2CH_3 , $OSO_2C_2H_5$, $OSO_2C_3H_7$, $OSO_2C_4H_9$, SH , SCH_3 , SC_2H_5 , SC_3H_7 , SC_4H_7 , SC_4H_9 , SC_5H_9 , SC_5H_{11} , SC_6H_{11} , SC_6H_{13} , SCF_3 , $SCOCH_3$, $SCOC_2H_5$, $SCOC_3H_7$, $SCOC_4H_9$, SO_3CH_3 , $SO_3C_2H_5$, $SO_3C_3H_7$, $SO_3C_4H_9$, SO_2NH , SO_2NH_2 , SO_2NHCH_3 , $SO_2N(CH_3)_2$, $SO_2NHC_2H_5$, $SO_2N(C_2H_5)_2$, $SO_2NHC_3H_7$, $SO_2N(C_3H_7)_2$, $SO_2NHC_4H_9$, $SO_2N(C_4H_9)_2$, NO_2 , CN , $COOCH_3$, $COOC_2H_5$, $COOC_3H_7$, $COOC_4H_9$,
20 $COSCH_3$, $COSC_2H_5$, $COSC_3H_7$, $COSC_4H_9$, $CONH_2$, $CONHCH_3$, $CON(CH_3)_2$, $CONHC_2H_5$, $CON(C_2H_5)_2$, $CONHC_3H_7$, $CON(C_3H_7)_2$, $CONHC_4H_9$, $CON(C_4H_9)_2$,

with the proviso that when either of R^8 or R^9 contain an aryl ring substituted at at least two adjacent positions on said aryl ring, then said two adjacent positions can together be selected from SCH_2S , SCH_2CH_2S ,
25 OCH_2O , or OCH_2CH_2O to form a bi-cyclic structure;

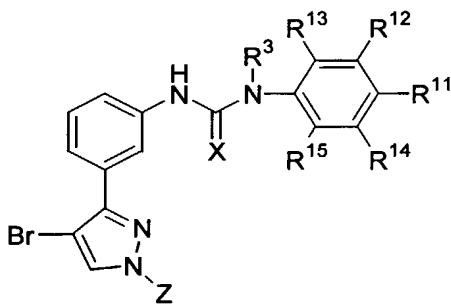
R^{12} , R^{13} , R^{14} , and R^{15} are each independently selected from the following: F, Cl, Br, I, CF_3 , CCl_3 , CH_3 , C_2H_5 , C_3H_7 , C_4H_9 , NH_2 , $NHCH_3$, $N(CH_3)_2$, NHC_2H_5 , $N(C_2H_5)_2$, NHC_3H_7 , $N(C_3H_7)_2$, NHC_4H_9 , $N(C_4H_9)_2$, $NHCOH$, $NHCOCH_3$, $NHCOC_2H_5$, $NHCOC_3H_7$, $NHCOC_4H_9$, $NHSO_2CH_3$, $NHSO_2C_2H_5$, $NHSO_2C_3H_7$, $NHSO_2C_4H_9$, OH , OCH_3 , OC_2H_5 ,
30 OC_3H_7 , OC_4H_7 , OC_4H_9 , OC_5H_9 , OC_5H_{11} , OC_6H_{11} , OC_6H_{13} , OCF_3 , $OCOCH_3$, $OCOC_2H_5$, $OCOC_3H_7$, $OCOC_4H_9$, OSO_2CH_3 , $OSO_2C_2H_5$, $OSO_2C_3H_7$, $OSO_2C_4H_9$, SH , SCH_3 , SC_2H_5 , SC_3H_7 , SC_4H_7 , SC_4H_9 , SC_5H_9 , SC_5H_{11} , SC_6H_{11} , SC_6H_{13} , SCF_3 , $SCOCH_3$, $SCOC_2H_5$, $SCOC_3H_7$, $SCOC_4H_9$, SO_3CH_3 , $SO_3C_2H_5$, $SO_3C_3H_7$, $SO_3C_4H_9$, SO_2NH , SO_2NH_2 ,

SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅, SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂,
 SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN, COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉,
 COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉, CONH₂, CONHCH₃, CON(CH₃)₂,
 CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂, CONHC₄H₉, CON(C₄H₉)₂, and

5 with the proviso that when any two adjacent positions of **R**¹², **R**¹³, **R**¹⁴, and
R¹⁵ are substituted, said two adjacent positions can together be further
 selected from SCH₂S, SCH₂CH₂S, OCH₂O, or OCH₂CH₂O to form a bi-
 cyclic structure; and

with the proviso that at least one of **R**¹², **R**¹³, **R**¹⁴, and **R**¹⁵ must be H.

10 More preferred compounds falling within the scope of Class (1) compounds are
 defined as follows:



15 wherein:

X is O or S;

Z is H or CH₃;

R³ and **R**¹⁰ are each independently selected from H, C₁₋₈ straight chain or branched
 20 alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl;

R¹¹ is H, F, Cl, Br, I, **R**⁷, CF₃, CCl₃, NR⁸R⁹, NR¹⁰COR⁷, NR¹⁰SO₂R⁷, OR⁷, OCF₃,
 OCOR⁷, OSO₂R⁷, SR⁷, SCF₃, SCOR⁷, SO₃R⁷, SO₂NR⁸R⁹, NO₂, CN, COOR⁷, COSR⁷,
 CONR⁸R⁹,

25 with the proviso that when a position adjacent to **R**¹¹ is substituted, then **R**¹¹
 and said adjacent position can together be selected from SCH₂S,
 SCH₂CH₂S, OCH₂O, or OCH₂CH₂O to form a bi-cyclic structure;

R⁷ is H, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl,

C₂₋₈ alkenyl, aryl or alkylaryl;

R⁸ and **R⁹** are independently selected from H, C₁₋₈ straight chain or branched alkyl, C₂₋₈ alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH₂aryl wherein each moiety within said C₁₋₈ straight chain or branched alkyl, C₂₋₈ alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH₂aryl may be optionally substituted by up to four substituents in any position, whereby each substituent is independently selected from: F, Cl, Br, I, CF₃, CCl₃, CH₃, C₂H₅, C₃H₇, C₄H₉, NH₂, NHCH₃, N(CH₃)₂, NHC₂H₅, N(C₂H₅)₂, NHC₃H₇, N(C₃H₇)₂, NHC₄H₉, N(C₄H₉)₂, NHCOH, NHCOCH₃, NHCOC₂H₅, NHCOC₃H₇, NHCOC₄H₉, NHSO₂CH₃, NHSO₂C₂H₅, NHSO₂C₃H₇, NHSO₂C₄H₉, OH, OCH₃, OC₂H₅, OC₃H₇, OC₄H₉, OC₅H₉, OC₅H₁₁, OC₆H₁₁, OC₆H₁₃, OCF₃, OCOCH₃, OCOC₂H₅, OCOC₃H₇, OCOC₄H₉, OSO₂CH₃, OSO₂C₂H₅, OSO₂C₃H₇, OSO₂C₄H₉, SH, SCH₃, SC₂H₅, SC₃H₇, SC₄H₉, SC₅H₉, SC₅H₁₁, SC₆H₁₁, SC₆H₁₃, SCF₃, SCOCH₃, SCOC₂H₅, SCOC₃H₇, SCOC₄H₉, SO₃CH₃, SO₃C₂H₅, SO₃C₃H₇, SO₃C₄H₉, SO₂NH, SO₂NH₂, SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅, SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂, SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN, COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉, COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉, CONH₂, CONHCH₃, CON(CH₃)₂, CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂, CONHC₄H₉, CON(C₄H₉)₂, and

with the proviso that when either of **R⁸** or **R⁹** contain an aryl ring substituted at at least two adjacent positions on said aryl ring, then said two adjacent positions can together be selected from SCH₂S, SCH₂CH₂S, OCH₂O, or OCH₂CH₂O to form a bi-cyclic structure;

R¹², **R¹³**, **R¹⁴**, and **R¹⁵** are each independently selected from: F, Cl, Br, I, CF₃, CCl₃, CH₃, C₂H₅, C₃H₇, C₄H₉, NH₂, NHCH₃, N(CH₃)₂, NHC₂H₅, N(C₂H₅)₂, NHC₃H₇, N(C₃H₇)₂, NHC₄H₉, N(C₄H₉)₂, NHCOH, NHCOCH₃, NHCOC₂H₅, NHCOC₃H₇, NHCOC₄H₉, NHSO₂CH₃, NHSO₂C₂H₅, NHSO₂C₃H₇, NHSO₂C₄H₉, OH, OCH₃, OC₂H₅, OC₃H₇, OC₄H₉, OC₅H₉, OC₅H₁₁, OC₆H₁₁, OC₆H₁₃, OCF₃, OCOCH₃, OCOC₂H₅, OCOC₃H₇, OCOC₄H₉, OSO₂CH₃, OSO₂C₂H₅, OSO₂C₃H₇, OSO₂C₄H₉, SH, SCH₃, SC₂H₅, SC₃H₇, SC₄H₉, SC₅H₉, SC₅H₁₁, SC₆H₁₁, SC₆H₁₃, SCF₃, SCOCH₃, SCOC₂H₅, SCOC₃H₇, SCOC₄H₉, SO₃CH₃, SO₃C₂H₅, SO₃C₃H₇, SO₃C₄H₉, SO₂NH, SO₂NH₂, SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅, SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂, SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN, COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉, COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉, CONH₂, CONHCH₃, CON(CH₃)₂, CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂, CONHC₄H₉, CON(C₄H₉)₂, and

with the proviso that when any two adjacent positions of R^{12} , R^{13} , R^{14} , and R^{15} are substituted, said two adjacent positions can together be further selected from SCH_2S , SCH_2CH_2S , OCH_2O , or OCH_2CH_2O to form a bi-cyclic structure; and

5 with the proviso that at least two of R^{12} , R^{13} , R^{14} , and R^{15} must be H.

Exemplary compounds of general Formula (A), Class (1) are set forth below. Based upon *in vivo* data developed (as set forth below), Compounds 116081 and 116082 are particularly preferred.

Inositol phosphate accumulation assays evidence the activity of test compounds. Both single concentration percentages of control values and IC_{50} determinations indicate activity. In the tables below the column legends have the following meanings:

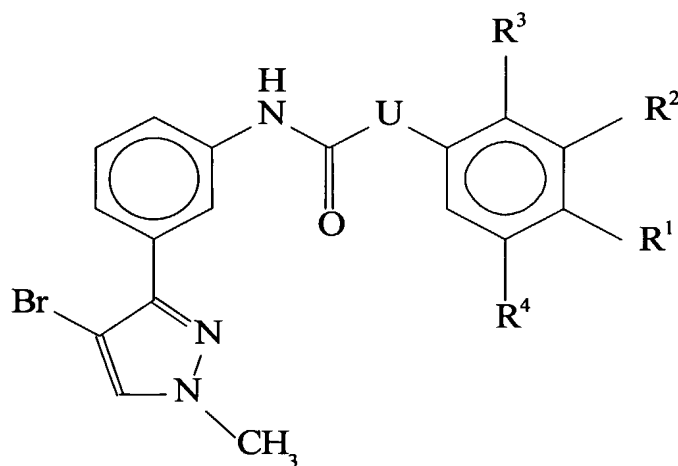
IP₃ % Control: The values in this column reflect an IP Accumulation Assay where the test compounds were evaluated at one concentration of 10 μ M. For these assays, the compound was diluted into inositol-free Dulbecco's Eagle Media containing 10 μ M pargyline and 10 mM LiCl and tested at a final assay concentration of 10 μ M, in triplicate. The percent control value was calculated based on the control in which no test compound was added.

IP₃ AP-3 IC_{50} nM: The values in this column reflect an IP accumulation assay in which the test compound was evaluated at several different concentrations whereby an IC_{50} could be determined. This column corresponds to the column appearing in the tables above which is labeled: Inositol Phosphate Accumulation, AP-3, IC_{50} Value (μ M).

WT 5HT_{2A} LSD IC_{50} nM: The values in this column reflect a competitive binding assay using LSD. This column corresponds to the column appearing in the tables above which is labeled: Competitive Binding, WT 5HT_{2A}, ($[^3H]$ LSD), IC_{50} Value (μ M).

(Note: A "dash" in the table indicates that no value was determined.)

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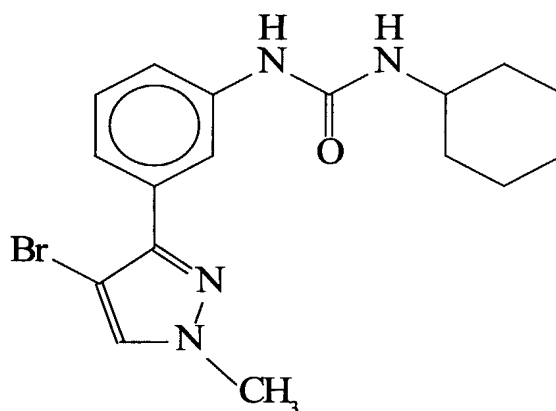


| Compound No. | R ¹ | R ² | R ³ | R ⁴ | X | U | IP ₃ % of Control | IP ₃ AP-3 IC ₅₀ nM | WT 5HT _{2A} LSD IC ₅₀ nM |
|---|------------------|----------------|-------------------|----------------|---|----|---------------------------------|--|---|
| N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][(4-methylthiophenyl)amino]carboxamide | | | | | | | | | |
| 116079 | SCH ₃ | H | H | H | O | NH | 16 | 17 | 4 |
| N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][(4-chlorophenyl)amino]carboxamide | | | | | | | | | |
| 116081 | Cl | H | H | H | O | NH | 10 | 3.2 | 11 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-fluorophenyl)carboxamide | | | | | | | | | |
| 116082 | F | H | H | H | O | NH | 11 | - | 7 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[2-(trifluoromethoxy)phenyl]carboxamide | | | | | | | | | |
| 116087 | H | H | CF ₃ O | H | O | NH | 11 | - | 200 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-nitrophenyl)carboxamide | | | | | | | | | |
| 116089 | H | H | NO ₂ | H | O | NH | 27 | - | 238 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-methoxyphenyl)carboxamide | | | | | | | | | |
| 116091 | MeO | H | H | H | O | NH | 12 | - | 19 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-methylphenyl)carboxamide | | | | | | | | | |
| 116092 | H | H | Me | H | O | NH | 32 | - | 131 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(trifluoromethyl)phenyl]carboxamide | | | | | | | | | |

| | | | | | | | | | |
|--|-------------------|-----|-------------------|----|---|------------------|----|-----|------|
| 116097 | CF ₃ | H | H | H | O | NH | 11 | - | 65 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-chlorophenyl)carboxamide | | | | | | | | | |
| 116105 | H | Cl | H | H | O | NH | 11 | - | 39 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-chlorophenyl)carboxamide | | | | | | | | | |
| 116108 | H | H | Cl | H | O | NH | 6 | - | 249 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(methylethyl)phenyl]carboxamide | | | | | | | | | |
| 116110 | isopropyl | H | H | H | O | NH | 7 | - | 338 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-methoxyphenyl)carboxamide | | | | | | | | | |
| 116111 | H | MeO | H | H | O | NH | 7 | - | 106 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-methylphenyl)carboxamide | | | | | | | | | |
| 116112 | H | Me | H | H | O | NH | 14 | - | 57 |
| [[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino]-N-methyl-N-[4-(trifluoromethoxy)phenyl]carboxamide | | | | | | | | | |
| 116113 | CF ₃ O | H | H | H | O | NCH ₃ | - | 193 | 2 |
| N-[4-(tert-butyl)phenyl]{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide | | | | | | | | | |
| 116119 | t-butyl | H | H | H | O | NH | 17 | - | 476 |
| N-[4-(dimethylamino)phenyl]{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide | | | | | | | | | |
| 116122 | NMe ₂ | H | H | H | O | NH | 9 | - | 309 |
| N-(3,5-dichloro-4-methylphenyl){[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide | | | | | | | | | |
| 116138 | Me | Cl | H | Cl | O | NH | 23 | - | 122 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(trifluoromethylthio)phenyl]carboxamide | | | | | | | | | |
| 116139 | CF ₃ S | H | H | H | O | NH | 12 | - | 56 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-fluorophenyl)carboxamide | | | | | | | | | |
| 116144 | H | H | F | H | O | NH | 12 | - | 37 |
| 2-([3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino)carbonylamino)benzamide | | | | | | | | | |
| 116145 | H | H | CONH ₂ | H | O | NH | 31 | - | 7473 |

| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-cyanophenyl)carboxamide | | | | | | | | | |
|--|----|---|----|---|---|----|----|---|-----|
| 116147 | CN | H | H | H | O | NH | 12 | - | 2 |
| {[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-cyanophenyl)carboxamide | | | | | | | | | |
| 116148 | H | H | CN | H | O | NH | 30 | - | 348 |

Additional compounds falling under the defined parameters of class (1) are as follows:



5

| Compound No. | N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][cyclohexylamino]carboxamide | IP ₃ AP-3 | WT 5HT _{2A} LSD |
|--------------|--|----------------------|--------------------------|
| | | IC ₅₀ nM | IC ₅₀ nM |
| 116141 | | 114 | 81 |

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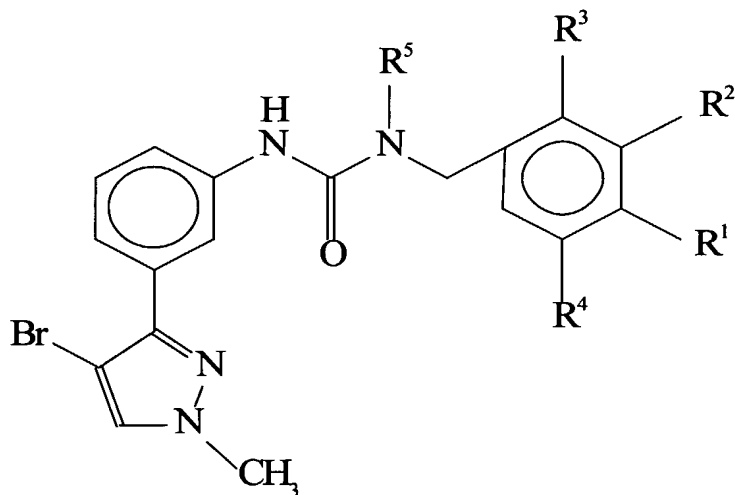
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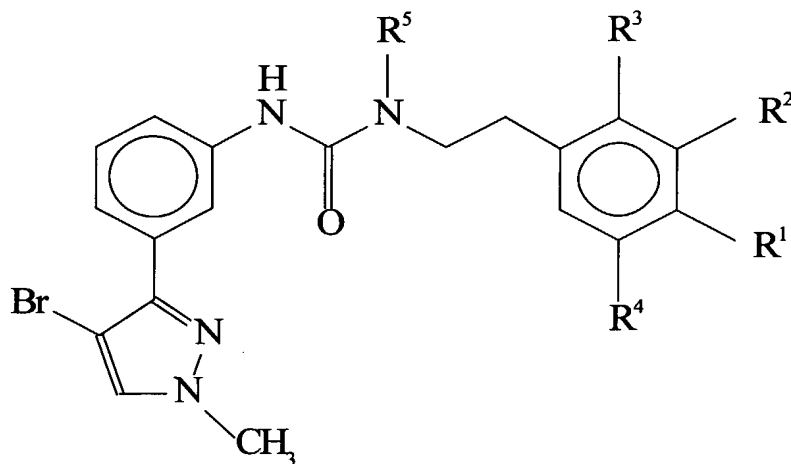
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| Compound No. | R ¹ | R ² | R ³ | R ⁴ | R ⁵ | IP ₃ AP-3 IC ₅₀ nM | WT 5HT _{2A} LSD IC ₅₀ nM |
|--|----------------|----------------|----------------|----------------|----------------|---|---|
| N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][phenylmethylamino]carboxamide | | | | | | | |
| 116143 | H | H | H | H | H | 120 | 47 |
| N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-fluorophenyl)methyl}amino]carboxamide | | | | | | | |
| 116182 | F | H | H | H | H | 89 | 132 |
| N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(3,4-dimethoxyphenyl)methyl}amino]carboxamide | | | | | | | |
| 116183 | OMe | OMe | H | H | H | - | 1010 |
| N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(3,4,5-trimethoxyphenyl)methyl}amino]carboxamide | | | | | | | |
| 116184 | OMe | OMe | H | OMe | H | - | 2960 |
| N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(2-methylphenyl)methyl}amino]carboxamide | | | | | | | |
| 116185 | H | H | Me | H | H | - | 769 |
| N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{(4-methoxyphenyl)methyl}amino]carboxamide | | | | | | | |
| 116189 | OMe | H | H | H | H | - | 102 |



| Compound No. | R ¹ | R ² | R ³ | R ⁴ | R ⁵ | IP ₃ AP-3 | WT 5HT _{2A} LSD |
|---|----------------|----------------|----------------|----------------|----------------|-------------------------|--------------------------------|
| | | | | | | IC ₅₀ nM | IC ₅₀ nM |
| N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][{2-(4-methoxyphenyl)ethyl} amino]carboxamide | | | | | | | |
| 116194 | OMe | H | H | H | H | 32 | 61 |

EXAMPLE 11

IN VIVO ANALYSIS

A. 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane ("DOI") Motor Dysfunction Analysis: 116081 and 116082

The profiles of 116082 and 116081 in *in vitro* functional assays suggested that this compound exhibit selective 5HT_{2A} inverse agonist properties. Assessment of 5-HT₂ receptor antagonism *in vivo* was made by using the DOI locomotor activity test in rats as described by Krebs-Thomson (*Psychopharmacology*, 140:69-74, 1998).

The peripheral administration of the hallucinogen and 5HT₂ agonist DOI [1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane] at a dose of 0.3 mg/kg typically produces motor dysfunction in rats as shown by a decrease in ambulation (*i.e.* walking and running), fine movement of the body at rest (*i.e.* grooming, licking) and rearing activity (*i.e.* standing on hindlimbs). Motor function was assessed by using automated locomotor activity cages. The rats were placed in a standard rodent cage surrounded by photocell beams, which allow for automated recording of motor activity. The animals were under

no motivational constraints and were free to move around the cage. In this test, male Sprague-Dawley rats (n = 6 per dose) were administered with 11081 or 11082 (intraperitoneal injection) followed by a subcutaneous administration of DOI 30 min later. A reversal of DOI-induced motor dysfunction in rats is considered to be predictive of the ability of the test compound to antagonize 5HT2 receptors *in vivo*.

Based upon the results presented in Figures 3 and 4, a conclusion that can be drawn from the data is that 116081 and 116082 beneficially reverses DOI-induced motor dysfunction. Based upon the data developed, another conclusion that can be drawn is that 116082 exhibits its serotonin 5HT2 antagonist properties at doses ranging from 5 to 10 mg/kg and that 116081 exhibits its serotonin 5HT2 antagonist properties at dose ranging from 2.5 to 10 mg/kg.

B. Extrapyramidal Side Effect Analysis: 116082

A significant problem of currently marketed typical antipsychotics such as haloperidol is the occurrence of extrapyramidal side effects. The extrapyramidal motor syndrome (EPS) is characterized by Parkinson-like symptoms resulting from the blockade of brain striatal dopamine D2 and D1 receptors (Snyder, SH. *Am. J. Psychiatry*, 138:461-468, 1981).

The propensity of a potential therapeutic to block striatal dopamine receptors can be evaluated by measurement of the induction of catalepsy in rodents (Hoffman and Donavan, *Psychopharmacology* 120:128-133, 1995). Catalepsy is characterized by body rigidity and is commonly measured using the bar test in rats (Prinssen et al., *Psychopharmacology*, 144:20-29,1999). Thus, this test was used to determine the potential EPS side effect liability of 116082 *in vivo*.

In the bar test, the rat's forelimbs were placed on a horizontal, cylindrical metal bar (diameter 0.75 cm, height 10 cm) and the time during which both forelimbs remained on the bar was determined up to a maximum of 30 sec. This test was repeated 3 times consecutively and catalepsy was defined as the average of the three duration measurements. Male Sprague-Dawley (average body weight 300 g) rats were administered 116082 (intraperitoneal injection) 60 min prior to the test. The number of animals tested at each doses was 6 to 8.

The data presented in Figure 5 support the conclusion that 116082 did not induce a dose-dependent increase in time spent on the bar, thus suggesting that 116082 does not induce catalepsy in rats at doses ranging from 10 to 80 mg/kg. On the other hand,

haloperidol induced a significant increase in time spent on the bar indicating that this compound induces catalepsy in rats at a dose of 0.8 mg/kg. The data thus support the conclusion that 116082 does not induce extra-pyramidal side effects in rats at a dose 8 times greater than the dose required to block 5HT_{2A} receptors *in vivo* (i.e., see DOI example, *supra*).
5

Using the same dosing parameters and protocol, it was further determined that 116081 also did not produce a cataleptic response, under the criteria set forth above (n=10 per dose). Data not shown.

C. Oral Availability: 116081 and 116082

10 Based upon the *in vivo* data developed, oral bioavailability of compounds 110681 and 110682 was considered. The compounds were administered by oral gavage at a dose of 50 mg/kg, 30 min prior to DOI in rats (see Example 11A, *supra*). Animals were placed in the locomotor activity cages 10 min after DOI administration and activity was measured for 10 min. Results, presented in Figure 6, provide total activity counts over the 10 min of exposure to the locomotor activity apparatus.
15

The data presented in Figure 6 support the conclusion that oral administration of 116082 and 116081 at a dose of 50 mg/kg reversed DOI-induced motor dysfunction in rats. The data further support the conclusion that a 50mg/kg dose of 116082 and 116081 administered orally was as efficacious as a 10mg/kg dose of the same compounds administered intraperitoneally (Figure 3 and 4).
20

These data support the conclusion that 116082 and 116081 are orally active, with oral bioavailability greater or equal to 20%.

D. Antagonism of MK801-induced hyperlocomotion: a model of potential antipsychotic ("antipositive") activity

25 In rodents, the non-competitive NMDA receptor antagonist MK-801 induces significant increases in locomotor activity and stereotypy. Because part of the symptomatology of schizophrenia may be related to altered glutamate transmission at the NMDA receptor, the reversal of MK-801-induced hyperlocomotor activity in rodents has been used routinely as an animal model for detecting potential antipsychotic activity (O'Neill et al., *Pharmacology Biochem. Behav.*, 63: 237-243, 1999). Thus, this test was
30 used to determine potential antipsychotic properties of 116081 *in vivo*.

Motor activity was assessed using automated locomotor activity cages as described above (Example 11(A), *supra*). Male Sprague-Dawley rats (250-350 g body weight) were

administered test compounds (i.p.) and placed in the locomotor activity cages for 30 min of habituation. Following habituation, animals were administered MK-801 (0.5 μ mol/kg = 0.17 mg/kg, s.c.) and then immediately placed back into the locomotor activity cages, and activity was measured for 120 min. A reversal of MK-801-induced motor activity (as shown by a significant increase of ambulation) in rats is considered to be predictive of the ability of the test compound to reverse psychotic symptoms. Results are presented in Figure 7.

Based upon the results presented in Figures 7A and 7B, 116081 appears to be equiefficacious to the atypical antipsychotic clozapine in reversing the motor activity induced by MK-801. The data, therefore, thus support the conclusion that AR116081 may have antipsychotic properties.

EXAMPLE 12

SYNTHETIC APPROACHES

The compounds disclosed in this invention may be readily prepared according to a variety of synthetic manipulations, all of which would be familiar to one skilled in the art. In the general syntheses set forth below, the labeled substituents have the same identifications as set out in the definitions of the compounds above.

Compounds of general formula (I) can be obtained *via* a variety of synthetic routes all of which would be familiar to one skilled in the art. The reaction of isocyanates with amines is a commonly practiced method for the formation of ureas (see Org. Syn. Coll. Vol. V, (1973), 555). Amine (IV), 3-(4-bromo-1-methylpyrazole-3-yl)phenylamine, commercially available from Maybridge Chemical Company, Catalog No. KM01978, CAS No. 175201-77-1] reacts readily with isocyanates (V) in inert solvents such as halocarbons to yield the desired ureas of general formula (I) wherein $R^1 = R^2 = H$:

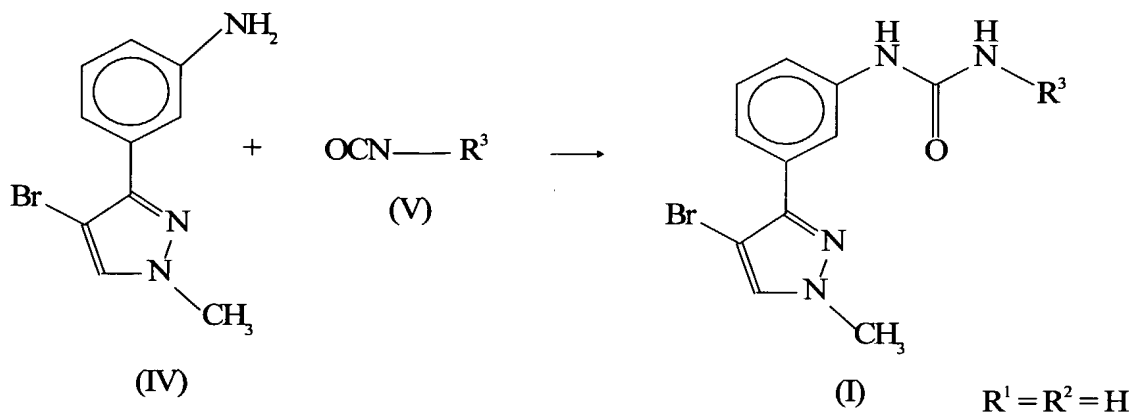
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Alternatively the amine (IV) can be converted to the corresponding isocyanate (VI) by the action of phosgene or a suitable phosgene equivalent, *e.g.* triphosgene, in an inert solvent such as a halocarbon in the presence of an organic base such as triethylamine or ethyldiisopropylamine. Isocyanate (VI) reacts with amines of general formula (VII), in an analogous fashion to that described above for the reaction of (IV) with (V), yielding the desired ureas of general formula (I) wherein $\text{R}^1 = \text{H}$:

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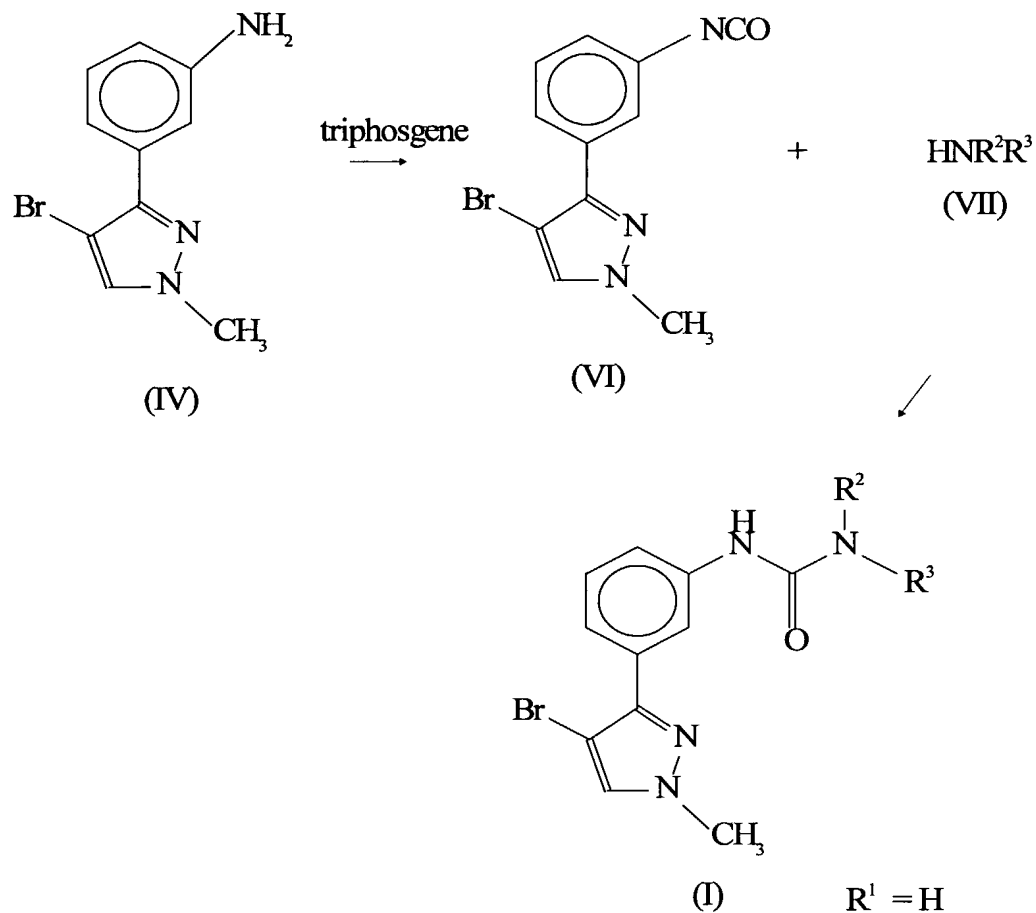
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Alternatively wherein the isocyanate of general formula (V) is not commercially available it can be prepared from the corresponding amine of general formula (VIII) in an analogous procedure to that described above for the preparation of (VI). Reaction of these isocyanates with (IV) would again yield the requisite ureas of general formula (I) wherein $\text{R}^1 = \text{R}^2 = \text{H}$:

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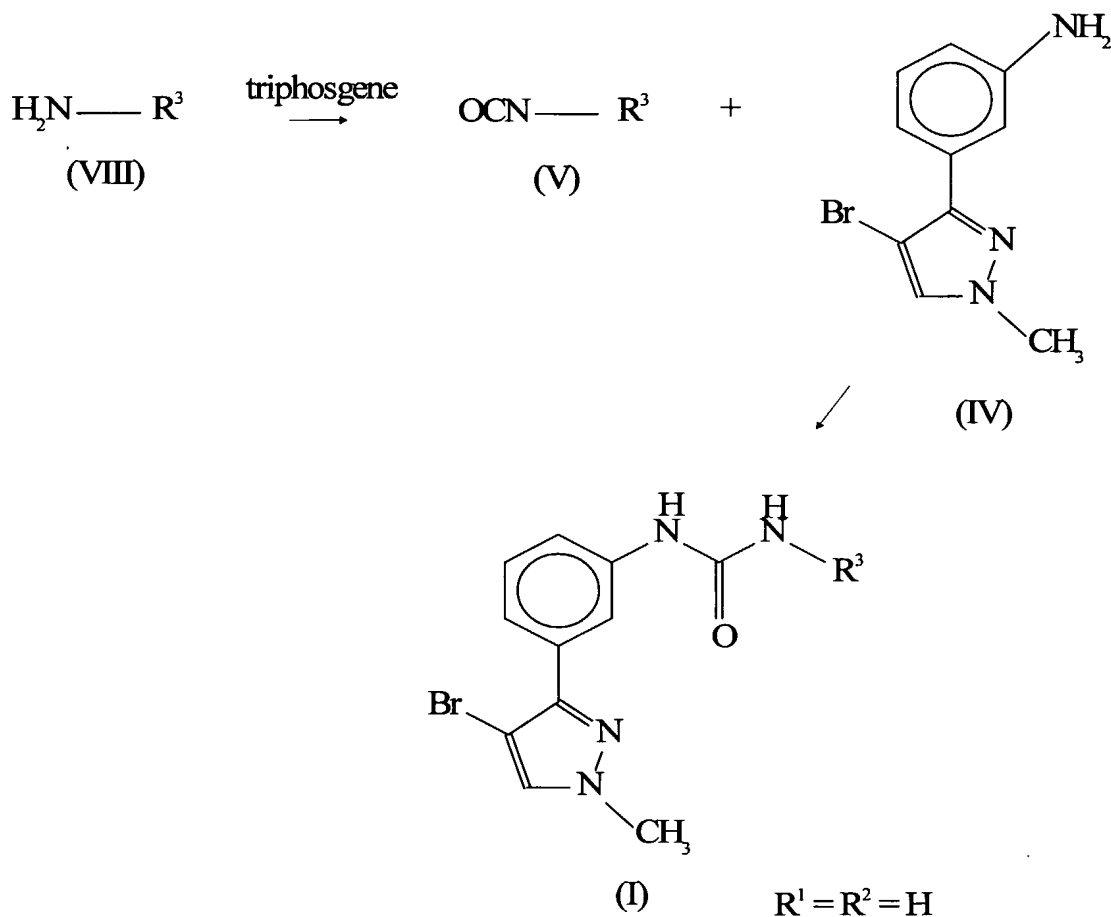
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Amines of general formula (VII) are also readily converted to activated isocyanate equivalents of general formula (IX) by the sequential action of carbonyldiimidazole and methyl iodide in tetrahydrofuran and acetonitrile respectively (R.A. Batey *et al*, *Tetrahedron Lett.*, (1998), 39, 6267-6270.) Reaction of (IX) with (IV) in an inert solvent such as a halocarbon would yield the requisite ureas of general formula (I) wherein $\text{R}^1 = \text{H}$:

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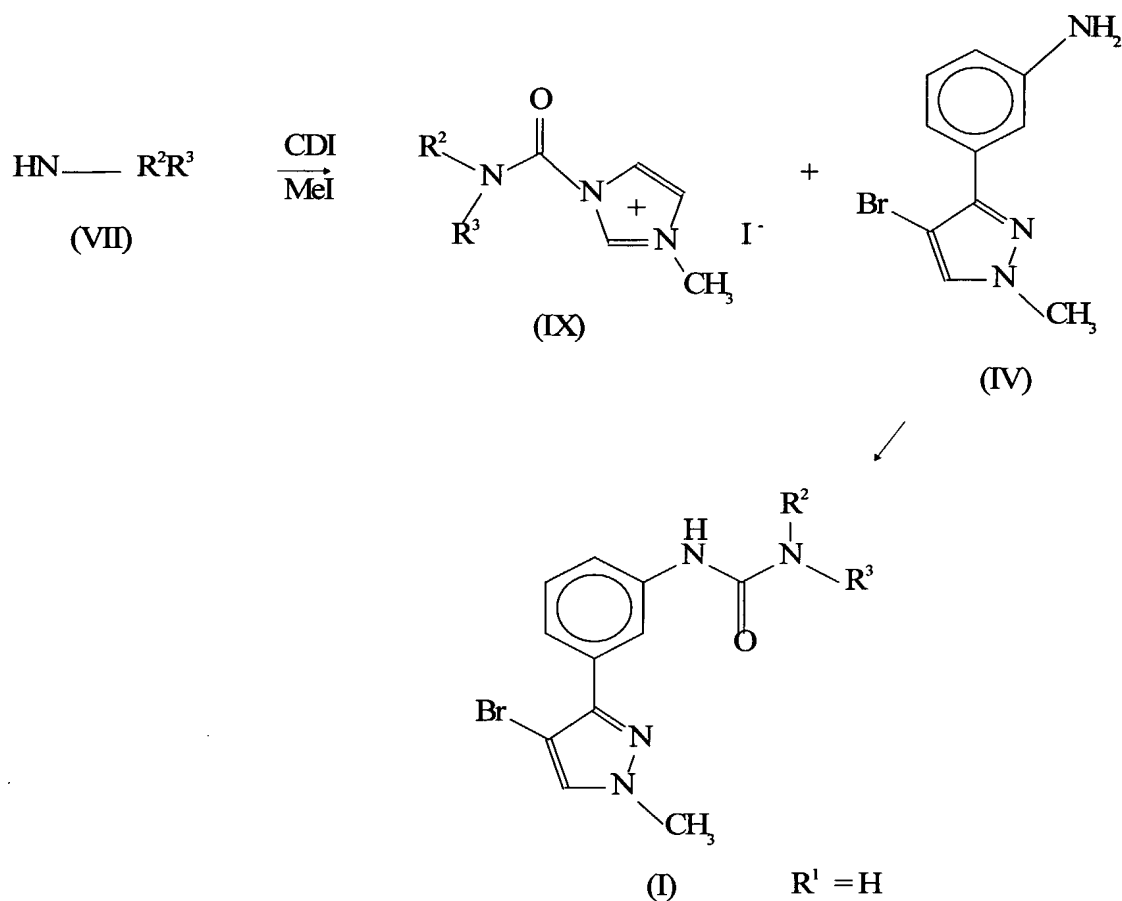
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Amine (IV) may be monomethylated according to the procedure of J. Barluenga *et al*, *J. Chem. Soc., Chem. Commun.*, (1984), 20, 1334-1335, or alkylated according to the procedure of P. Marchini *et al*, *J. Org. Chem.*, (1975), 40(23), 3453-3456, to yield compounds of general formula (X) wherein $\text{R}^1 = \text{lower alkyl}$. These materials may be reacted as above with reagents of general formula (V) and (IX) as depicted below:

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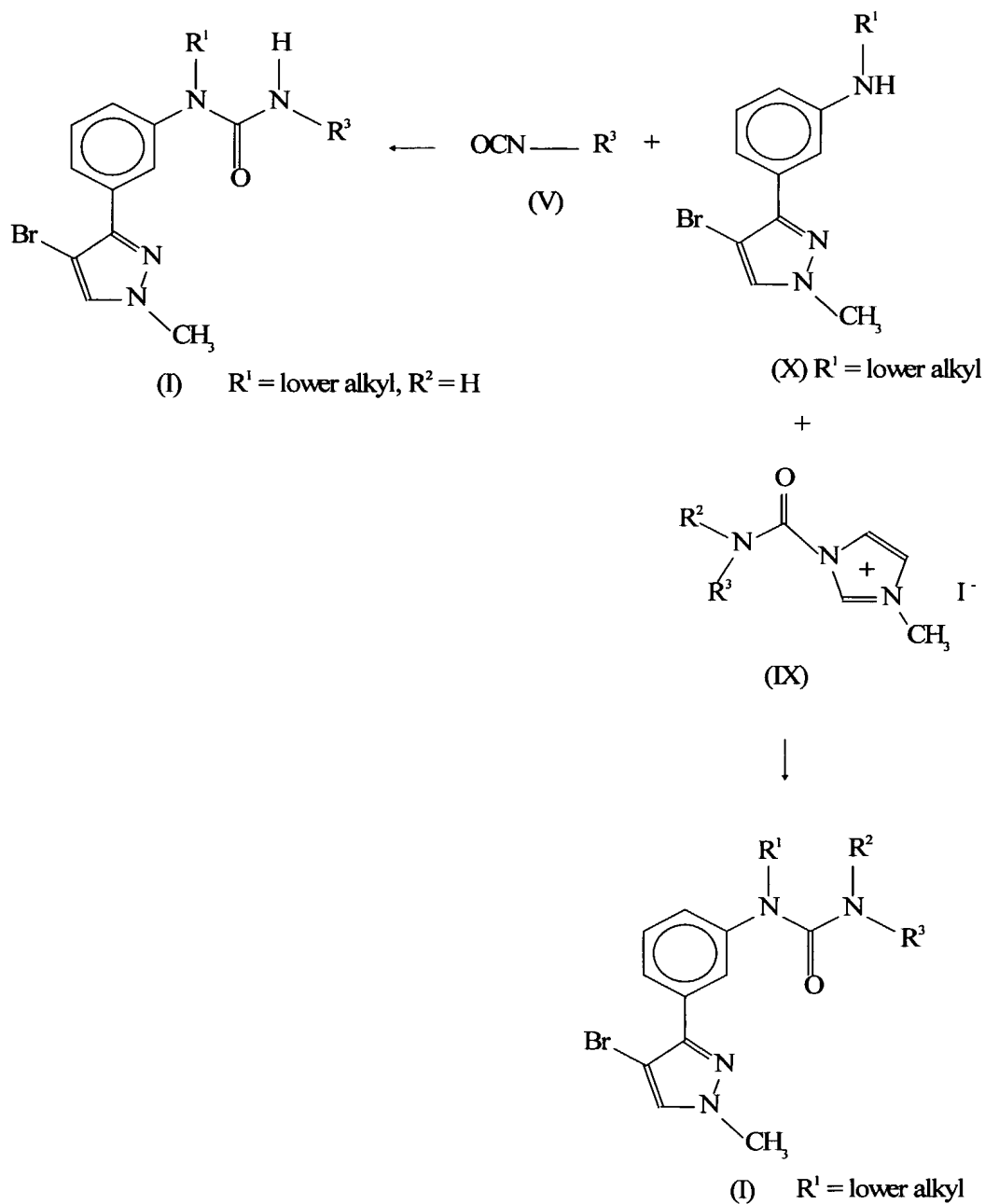
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Compounds of formula (A) or a solvate or physiologically functional derivative thereof for use as a therapeutic agent, specifically as a modifier of the activity of the serotonin 5-HT_{2A} receptor. Modifiers of the activity of the serotonin 5-HT_{2A} receptor are believed to be of potential use for the treatment or prophylaxis of CNS, gastrointestinal,

cardiovascular, and inflammatory disorders. Compounds of the formula (A) may be administered by oral, sublingual, parenteral, rectal, or topical administration. In addition to the neutral forms of compounds of formula (A) by appropriate addition of an ionizable substituent, which does not alter the receptor specificity of the compound, physiologically acceptable salts of the compounds may also be formed and used as therapeutic agents. Different amounts of the compounds of formula (A) will be required to achieve the desired biological effect. The amount will depend on factors such as the specific compound, the use for which it is intended, the means of administration, and the condition of the treated individual. A typical dose may be expected to fall in the range of 0.001 to 200 mg per kilogram of body weight of the treated individual. Unit does may contain from 1 to 200 mg of the compounds of formula (A) and may be administered one or more times a day, individually or in multiples. In the case of the salt or solvate of a compound of formulas (A), the dose is based on the cation (for salts) or the unsolvated compound.

Compositions, including, but not limited to, pharmaceutical compositions, comprising at least one compound of formula (A) and/or an acceptable salt or solvate thereof (*e.g.*, a pharmaceutically acceptable salt or solvate) as an active ingredient combined with at least one carrier or excipient (*e.g.*, pharmaceutical carrier or excipient). Pharmaceutical compositions may be used in the treatment of clinical conditions for which a modifier of the activity of the serotonin 5-HT_{2A} receptor is indicated, particularly where the active ingredient is preferentially selective for the 5HT_{2A} receptor over the 5HT_{2A} receptor, and most particularly where the active ingredient is also an inverse agonist at the 5HT_{2A} receptor. At least one compound of formula (A) may be combined with the carrier in either solid or liquid form in a unit dose formulation. The pharmaceutical carrier must be compatible with the other ingredients in the composition and must be tolerated by the individual recipient. Other physiologically active ingredients may be incorporated into the pharmaceutical composition of the invention if desired, and if such ingredients are compatible with the other ingredients in the composition. Formulations may be prepared by any suitable method, typically by uniformly mixing the active compound(s) with liquids or finely divided solid carriers, or both, in the required proportions, and then, if necessary, forming the resulting mixture into a desired shape.

Conventional excipients, such as binding agents, fillers, acceptable wetting agents, tableting lubricants, and disintegrants may be used in tablets and capsules for oral administration. Liquid preparations for oral administration may be in the form of solutions,

emulsions, aqueous or oily suspensions, and syrups. Alternatively, the oral preparations may be in the form of dry powder that can be reconstituted with water or another suitable liquid vehicle before use. Additional additives such as suspending or emulsifying agents, non-aqueous vehicles (including edible oils), preservatives, and flavorings and colorants may be added to the liquid preparations. Parenteral dosage forms may be prepared by dissolving the compound of the invention in a suitable liquid vehicle and filter sterilizing the solution before filling and sealing an appropriate vial or ampoule. These are just a few examples of the many appropriate methods well known in the art for preparing dosage forms.

The fifth aspect of the present invention provides for the use of a compound of formula (A) in the preparation of a medicament for the treatment of a medical condition for which a modifier of the activity of the serotonin 5-HT_{2A} receptor is indicated.

Another aspect of the present invention provides for a method of treatment of a clinical condition of a mammal, such as a human, for which a modifier of the activity of the serotonin 5-HT_{2A} receptor is indicated, which comprises the administration to the mammal of a therapeutically effective amount of a compound of formula (A) or a physiologically acceptable salt, solvate, or physiologically functional derivative thereof.

EXAMPLE 13

EXPERIMENTAL DATA

Mass spectra were recorded on a Micromass Platform™ LC with Gilson HPLC. Infra-red spectra were recorded on a Nicolet Avatar™ 360 FT-IR. Melting points were recorded on a Electrothermal IA9200™ apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded on a Bruker™ 300MHz machine. Chemical shifts are given with respect to tetramethylsilane. In the text the following abbreviations are used; s (singlet), d (doublet), t (triplet), m (multiplet) or combinations thereof. Chemical shifts are quoted in parts per million (ppm) and with coupling constants in Hertz.

Thin layer chromatography was carried out using aluminium backed silica plates (250μL; GF₂₅₄). HPLC was recorded either on a HP Chemstation™ 1100 HPLC using a Hichrom 3.5 C18 reverse phase column (50mm x 2.1mm i.d.). Linear gradient elution over 5 minutes – 95% water (+0.1% TFA) / 5% acetonitrile (+0.05% TFA) down to 5% water / 95% acetonitrile. Flow rate 0.8mL/min [Method A]; or on a Hichrom 3.5 C18 reverse phase column (100mm x 3.2mm i.d.). Linear gradient elution over 11 minutes – 95% water (+0.1% TFA) / 5% acetonitrile (+0.05% TFA) down to 5% water / 95% acetonitrile.

Flow rate 1mL/min [Method B]. Samples were routinely monitored at 254nm unless otherwise stated.

All reagents were purchased from commercial sources.

Experiment 1

5 Preparation and Analysis of 103487

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl] [(4-trifluoromethoxy)phenyl] amino] carboxamide

This compound is commercially available from Maybridge Chemical Company,
10 Catalog No. KM04515.

One or the other (as indicated) of the two following synthetic protocols was used to generate each of the compounds below:

Protocol A:

To an isocyanate (1mmol) in CH₂Cl₂ (4mL) was added dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (1mmol) in CH₂Cl₂ (4mL). The mixture was
15 stirred for 16 hours and concentrated. Chromatography on flash silica (20%-80% EtOAc/hexane) followed by recrystallisation gave the pure urea.

Protocol B:

To a stirred solution of triphosgene (0.33mmol) in CH₂Cl₂ (4mL) was added
20 dropwise a solution of 3-(3-aminophenyl)-4-bromo-1-methylpyrazole (1mmol) and triethylamine (2mmol) in CH₂Cl₂ (4mL). After 1 hour, an aniline was added (1mmol). The reaction mixture was stirred for 16 hours and concentrated. Chromatography on flash silica (20%-80%EtOAc/hexane) followed by recrystallisation gave the pure urea.

25 Experiment 2

Preparation and Analysis of 116079

N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl] [(4-methylthiophenyl) amino] carboxamide

[Protocol A] – 4-(methylthio)phenyl isocyanate

30 colourless solid (EtOAc/hexane)

MS (ES+): *m/z* (%) = 419 (M+H ⁸¹Br, 100), 417 (M+H ⁷⁹Br, 94).

¹H-NMR (MeOH d₄): δ = 2.42 (3H, s, SCH₃), 3.81 (3H, s, NCH₃), 7.06 (1H, m, ArH), 7.22 (2H, m, ArH), 7.37 (2H, m, ArH), 7.42-7.61 (4H, m, ArH).

HPLC: retention time 3.35 min [Method A].

Experiment 3**Preparation and Analysis of 116081****N-[3-(4-bromo-1-methylpyrazol-3-yl)phenyl][(4-chlorophenyl)amino]carboxamide**

5 [Protocol A] – 4-chlorophenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 409 (M+H ^{81}Br ^{37}Cl , 19), 407 (M+H ^{79}Br ^{37}Cl (^{81}Br ^{35}Cl), 100), 405 (M+H ^{79}Br ^{35}Cl , 81).

$^1\text{H-NMR}$ (MeOH d_4): δ = 3.81 (3H, s, CH_3), 7.07 (1H, m, ArH), 7.23 (2H, m, ArH), 7.36-7.60 (6H, m, ArH).

HPLC: retention time 3.42 min [Method A].

Experiment 4**Preparation and Analysis of 116082**

15 **{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-fluorophenyl)carboxamide**

[Protocol A] – 4-fluorophenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 391 (M+H ^{81}Br , 96), 389 (M+H ^{79}Br , 100).

20 $^1\text{H-NMR}$ (MeOH d_4): δ = 3.81 (3H, s, CH_3), 6.93-7.11 (3H, m, ArH), 7.37-7.61 (6H, m, ArH).

HPLC: retention time 3.11 min.

Experiment 5

25 **Preparation and Analysis of 116087**

{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[2-(trifluoromethoxy)phenyl]carboxamide

[Protocol A] - 2-(trifluoromethoxy)phenyl isocyanate

30 colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 457 (M+H ^{81}Br , 100), 455 (M+H ^{79}Br , 95).

$^1\text{H-NMR}$ (DMSO d_6): δ = 3.79 (3H, s, CH_3), 7.06-7.18 (2H, m, ArH), 7.38-7.49 (2H, m, ArH), 7.51-7.62 (2H, m, ArH), 7.65 (1H, m, ArH), 7.71 (1H, s, ArH), 8.24 (1H, dd, $J=1.1, 8.2$, ArH), 8.56 (1H, s, NH), 9.49 (1H, s, NH).

35 HPLC: retention time 3.40 min.

Experiment 6

Preparation and Analysis of 116089**{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-nitrophenyl)carboxamide**

[Protocol A] - 2-nitrophenyl isocyanate

5 yellow solid (EtOAc/hexane)

MS (ES+): m/z (%) = 418 (M+H ⁸¹Br, 98), 416 (M+H ⁷⁹Br, 100).

¹H-NMR (DMSO d₆): δ = ¹H-NMR (DMSO d₆): \square = 3.79 (3H, s, NCH₃), 7.14 (1H, m, ArH), 7.24 (1H, m, ArH), 7.50 (1H, t, J=7.7, ArH), 7.60 (2H, m, ArH), 7.67 (1H, s, ArH), 7.71 (1H, s, ArH), 8.10 (1H, m, ArH), 8.29 (1H, m, ArH), 9.65 (1H, s, NH), 10.09 (1H, s, NH).

10

HPLC: retention time 3.10 min [Method A].

Experiment 7**Preparation and Analysis of 116091**15 **{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-methoxyphenyl)carboxamide**

[Protocol A] – 4-methoxyphenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 403 (M+H ⁸¹Br, 100), 401 (M+H ⁷⁹Br, 96).

¹H-NMR (DMSO d₆): δ = 3.71 (3H, s, OCH₃), 3.79 (3H, s, NCH₃), 6.87 (2H, d, J=8.9, ArH), 7.06 (1H, d, J=7.5, ArH), 7.39 (2H, d, J=8.9, ArH), 7.45-7.61 (3H, m, ArH), 7.65 (1H, s, ArH), 8.52 (1H, s, NH), 8.84 (1H, s, NH).

20

HPLC: retention time 3.08 min.

25 **Experiment 8****Preparation and Analysis of 116092****{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-methylphenyl)carboxamide**[Protocol A] – *o*-tolyl isocyanate

30 colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 387 (M+H ⁸¹Br, 94), 385 (M+H ⁷⁹Br, 100).

¹H-NMR (MeOH d₄): δ = 2.29 (3H, s, CH₃), 3.81 (3H, s, NCH₃), 7.03 (1H, dt, J=1.1, 7.5, ArH), 7.09 (1H, dt, J=1.1, 7.5, ArH), 7.13-7.22 (2H, m, ArH), 7.45 (1H, t, J=7.9, ArH), 7.49-7.57 (2H, m, ArH), 7.60-7.68 (2H, m, ArH).

35 HPLC: retention time 2.96 min.

Experiment 9**Preparation and Analysis of 116097****{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(trifluoromethyl)phenyl]carboxamide**

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[Protocol A] – 4-(trifluoromethyl)phenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 441 (M+H ^{81}Br , 94), 439 (M+H ^{79}Br , 100). $^1\text{H-NMR}$ (MeOH d_4): δ = 3.82 (3H, s, CH_3), 7.04-7.16 (3H, m, ArH), 7.20-7.47

10 (6H, m, ArH).

HPLC: retention time 3.56 min.

Experiment 10**Preparation and Analysis of 116105**15 **{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-chlorophenyl)carboxamide**

[Protocol A] – 3-chlorophenyl isocyanate

colourless solid (EtOAc/hexane)

20 MS (ES+): m/z (%) = 409 (M+H ^{81}Br ^{37}Cl , 26), 407 (M+H ^{79}Br ^{37}Cl (^{81}Br ^{35}Cl), 100), 405 (M+H ^{79}Br ^{35}Cl , 70). $^1\text{H-NMR}$ (MeOH d_4): δ = 3.81 (3H, s, NCH_3), 7.04 (1H, m, ArH), 7.10 (1H, m, ArH), 7.28 (2H, m, ArH), 7.47 (1H, t, $J=7.8$, ArH), 7.55 (1H, m, ArH), 7.63 (1H, m, ArH), 7.68 (1H, s, ArH), 7.73 (1H, m, ArH), 9.04 (2H, s, NH).

HPLC: retention time 3.20 min [Method A].

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Experiment 11**Preparation and Analysis of 116108****{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-chlorophenyl)carboxamide**

30 [Protocol A] – 2-chlorophenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 409 (M+H ^{81}Br ^{37}Cl , 24), 407 (M+H ^{79}Br ^{37}Cl (^{81}Br ^{35}Cl), 100), 405 (M+H ^{79}Br ^{35}Cl , 72).35 $^1\text{H-NMR}$ (MeOH d_4): δ = 3.81 (3H, s, NCH_3), 7.03 (1H, m, ArH), 7.11 (1H, m, ArH), 7.28 (1H, m, ArH), 7.35-7.53 (3H, m, ArH), 7.55 (1H, s, ArH), 7.62 (1H, m, ArH), 8.11 (1H, m, ArH).

HPLC: retention time 3.13 min.

Experiment 12**Preparation and Analysis of 116110****{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(methylethyl)phenyl]carboxamide**

5 [Protocol A] – 4-isopropylphenyl isocyanate

colourless solid (THF/hexane)

MS (ES+): m/z (%) = 415 (M+H ^{81}Br , 100), 413 (M+H ^{79}Br , 92).

$^1\text{H-NMR}$ (MeOH d_4): δ = 1.23 (6H, d, $J=6.8$, $2\times\text{CH}_3$), 2.86 (1H, septet, $J=6.8$, CH), 3.82 (3H, s, NCH_3), 7.09 (1H, m, ArH), 7.16 (2H, d, $J=7.6$, ArH), 7.31 (2H, d, $J=7.6$, ArH), 7.42-7.51 (2H, m, ArH), 7.54 (1H, s, ArH), 7.59 (1H, m, ArH).

HPLC: retention time 3.66 min.

Experiment 13**Preparation and Analysis of 116111**15 **{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-methoxyphenyl)carboxamide**

[Protocol A] – 3-methoxyphenyl isocyanate

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 403 (M+H ^{81}Br , 100), 401 (M+H ^{79}Br , 96).

$^1\text{H-NMR}$ (MeOH d_4): δ = 3.73 (3H, s, OCH_3), 3.81 (3H, s, NCH_3), 6.59 (1H, m, ArH), 6.91 (1H, m, ArH), 7.08 (1H, m, ArH), 7.14 (2H, m, ArH), 7.39-7.61 (4H, m, ArH).

HPLC: retention time 2.90 min.

Experiment 1425 **Preparation and Analysis of 116112****{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3-methylphenyl)carboxamide**[Protocol A] – *m*-tolyl isocyanate

colourless solid (EtOAc/hexane)

30 MS (ES+): m/z (%) = 387 (M+H ^{81}Br , 100), 385 (M+H ^{79}Br , 96).

$^1\text{H-NMR}$ (DMSO d_6): δ = 2.26 (3H, s, CH_3), 3.76 (3H, s, NCH_3), 6.79 (1H, m, ArH), 7.06-7.22 (3H, m, ArH), 7.29 (1H, m, ArH), 7.43-7.62 (3H, m, ArH), 7.68 (1H, s, ArH), 8.65 (1H, s, NH), 8.89 (1H, s, NH).

HPLC: retention time 3.05 min [Method A].

Experiment 15**Preparation and Analysis of 116113**

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-methyl-N-[4-(trifluoromethoxy)phenyl]carboxamide

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[Protocol B] – *N*-methyl-4-(trifluoromethoxy)aniline

pale yellow solid (EtOAc/hexane)

MS (ES+): m/z (%) = 471 (M+H ^{81}Br , 88), 469 (M+H ^{79}Br , 100).

$^1\text{H-NMR}$ (MeOH d_4): δ = 3.35 (3H, s, NCH₃), 3.81 (3H, s, NCH₃), 7.09 (1H, m, ArH), 7.25-7.51 (8H, m, ArH).

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HPLC: retention time 3.56 min [Method A].

Experiment 16**Preparation and Analysis of 116119**

N-[4-(*tert*-butyl)phenyl]{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide

15

[Protocol B] – 4-*tert*-butylaniline

colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 429 (M+H ^{81}Br , 98), 427 (M+H ^{79}Br , 100).

$^1\text{H-NMR}$ (DMSO d_6): δ = 1.27 (9H, s, 3xCH₃), 3.79 (3H, s, NCH₃), 7.07 (1H, d, J=7.5, ArH), 7.29 (2H, d, J=8.7, ArH), 7.37 (2H, d, J=8.7, ArH), 7.45 (1H, t, J=7.5, ArH), 7.51-7.60 (2H, m, ArH), 7.66 (1H, s, ArH), 8.65 (1H, s, NH), 8.83 (1H, s, NH).

20

HPLC: retention time 3.77 min.

Experiment 17**Preparation and Analysis of 116122**

N-[4-(dimethylamino)phenyl]{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide

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[Protocol B] – *N,N*-dimethyl-*p*-phenylenediamine

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colourless solid (EtOAc/hexane)

MS (ES+): m/z (%) = 416 (M+H ^{81}Br , 96), 414 (M+H ^{79}Br , 100).

$^1\text{H-NMR}$ (DMSO d_6): δ = 2.86 (6H, s, NCH₃), 3.80 (3H, s, NCH₃), 6.80 (2H, m, ArH), 7.09 (1H, d, J=7.7, ArH), 7.28 (2H, m, ArH), 7.42 (1H, t, J=7.8, ArH), 7.52 (1H, m, ArH), 7.59 (1H, s, ArH), 7.67 (1H, s, ArH), 8.45 (1H, s, NH), 8.75 (1H, s, NH).

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HPLC: retention time 2.07 min [Method A].

Experiment 18

Preparation and Analysis of 116138**N-(3,5-dichloro-4-methylphenyl){[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carboxamide**

5 [Protocol B] – 3,5-dichloro-4-methylphenylamine

colourless solid (EtOAc/hexane)

MS (ES⁺): *m/z* (%) = 457 (M+H, 35), 455 (M+H, 100), 453 (M+H, 65).

¹H-NMR (DMSO *d*₆): δ = 2.32 (3H, s, CH₃), 3.79 (3H, s, NCH₃), 7.11 (1H, d, J=7.4, ArH), 7.46 (1H, t, J=7.8, ArH), 7.50-7.64 (4H, m, ArH), 7.68 (1H, s, ArH), 9.02
10 (1H, s, NH), 9.09 (1H, s, NH).

HPLC: retention time 3.66 min.

Experiment 19**Preparation and Analysis of 116139**

15 **{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[4-(trifluoromethylthio)phenyl]carboxamide**

[Protocol B] – 4-(trifluoromethylthio)aniline

colourless solid (EtOAc/hexane)

20 MS (ES⁺): *m/z* (%) = 473 (M+H ⁸¹Br, 100), 471 (M+H ⁷⁹Br, 94).

¹H-NMR (DMSO *d*₆): δ = 3.81 (3H, s, NCH₃), 7.11 (1H, d, J=7.5, ArH), 7.47 (1H, t, J=7.9, ArH), 7.51-7.63 (6H, m, ArH), 7.66 (1H, s, ArH), 9.03 (1H, s, NH), 9.16 (1H, s, NH).

HPLC: retention time 3.76 min.

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Experiment 20**Preparation and Analysis of 116141**

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(cyclohexyl)carboxamide

30 [Protocol B] – cyclohexylamine

colourless solid, m.p. 155.5-156.3°C (EtOAc/hexane).

MS (ES⁺): *m/z* (%) = 379 (M+H ⁸¹Br, 93), 377 (M+H ⁷⁹Br, 100).

¹H-NMR (DMSO *d*₆): δ = 1.07-1.34 (5H, m, 5xCH), 1.52 (1H, m, CH), 1.63 (2H, m, 2xCH), 1.76 (2H, m, 2xCH), 3.48 (1H, m, NCH), 3.74 (3H, s, CH₃), 6.15 (1H, d, J=7.8, ArH), 6.98 (1H, d, J=7.5, ArH), 7.32-7.43 (2H, m, ArH), 7.51 (1H, m, NH), 7.62 (1H, s, ArH), 8.50 (1H, s, NH).

35

HPLC: retention time 3.16 min [Method A].

TLC: retention factor 0.35 (50% EtOAc/hexane).

Experiment 21

Preparation and Analysis of 116143

5 **{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(phenylmethyl)carboxamide**

[Protocol B] – benzylamine

colourless solid, m.p. 144.5-146.2°C (EtOAc/hexane).

IR: ν_{\max} = 1622, 1565, 1467, 1374, 1239, 973, 802, 752, 695 cm^{-1} .

10 MS (ES+): m/z (%) = 387 (M+H ^{81}Br , 89), 385 (M+H ^{79}Br , 100).

$^1\text{H-NMR}$ (CD_3OD): δ = 3.81 (3H, s, CH_3), 4.40 (2H, s, CH_2), 7.05 (1H, m, ArH),
7.19-7.51 (9H, m, ArH).

HPLC: retention time 3.06 min [Method A].a

15 **Experiment 22**

Preparation and Analysis of 116144

{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-fluorophenyl)carboxamide

[Protocol A] – 2-fluorophenyl isocyanate

20 colourless solid (DCM/hexane)

MS (ES+): m/z (%) = 391 (M+H ^{81}Br , 100), 389 (M+H ^{79}Br , 90).

$^1\text{H-NMR}$ ($\text{MeOH } d_4$): δ = 3.79 (3H, s, NCH_3), 7.00-7.11 (4H, m, ArH), 7.40-7.56
(3H, m, ArH), 7.61 (1H, m, ArH), 8.09 (1H, m, ArH).

HPLC: retention time 3.01 min.

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Experiment 23

30 **Preparation and Analysis of 116145**

2-({[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}carbonylamino)benzamide

[Protocol B] – 2-aminobenzamide

colourless solid (EtOAc/hexane)

35 MS (ES+): m/z (%) = 399 (M+H -17 ^{81}Br , 100), 397 (M+H - 17 ^{79}Br , 94).

$^1\text{H-NMR}$ ($\text{DMSO } d_6$): δ = 3.79 (3H, s, NCH_3), 6.93-7.10 (2H, m, ArH), 7.45 (2H, t,
 $J=7.8$, ArH), 7.59-7.72 (5H, m, ArH), 8.22 (2H, m), 9.92 (1H, s, NH), 10.69 (1H, s, NH).

HPLC: retention time 2.88 min.

Experiment 24

Preparation and Analysis of 116147

5 **{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-cyanophenyl)carboxamide**

[Protocol B] – 4-aminobenzonitrile

colourless solid (EtOAc/hexane)

MS (ES⁺): m/z (%) = 398 (M+H ⁸¹Br, 100), 396 (M+H ⁷⁹Br, 96).

10 ¹H-NMR (MeOH d₄): δ = 3.81 (3H, s, NCH₃), 7.12 (1H, m, ArH), 7.46-7.57 (3H, m, ArH), 7.62-7.69 (5H, m, ArH).

HPLC: retention time 3.12 min.

Experiment 25

15 **Preparation and Analysis of 116148**

{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-cyanophenyl)carboxamide

[Protocol B] – 2-aminobenzonitrile

colourless solid (EtOAc/hexane)

MS (ES⁺): m/z (%) = 398 (M+H ⁸¹Br, 95), 396 (M+H ⁷⁹Br, 100).

20 ¹H-NMR (CDCl₃): δ = 3.79 (3H, s, CH₃), 7.13-7.28 (2H, m, ArH), 7.49 (1H, t, J=7.8, ArH), 7.57 (1H, m, ArH), 7.62 (1H, m, ArH), 7.65-7.71 (2H, m, ArH), 7.78 (1H, m, ArH), 8.07 (1H, d, J=8.6, ArH), 8.83 (1H, s, NH), 9.62 (1H, s, NH).

HPLC: retention time 3.05 min [Method A].

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Experiment 26

Preparation and Analysis of 116182

30 **{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-fluorophenylmethyl)carboxamide**

[Protocol B] – 4-fluorobenzylamine

colourless solid, m.p. 185.5-186.6°C (EtOAc/hexane).

MS (ES⁺): m/z (%) = 405 (M+H ⁸¹Br, 97), 403 (M+H ⁷⁹Br, 100).

¹H-NMR (DMSO d₆): δ = 3.75 (3H, s, CH₃), 4.28 (2H, d, J=6.0, CH₂), 6.73 (1H, t, J=5.9, NH), 7.01 (1H, d, J=7.5, ArH), 7.10-7.18 (2H, m, ArH), 7.27-7.41 (4H, m, ArH), 7.56 (1H, s, ArH), 7.62 (1H, s, ArH), 8.82 (1H, s, NH).

HPLC: retention time 3.10 min [Method A].

5 TLC: retention factor 0.25 (50% EtOAc/hexane).

Experiment 27

Preparation and Analysis of 116183

10 **{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3,4-dimethoxyphenylmethyl)carboxamide**

[Protocol B] – 3,4-dimethoxybenzylamine

colourless solid, m.p. 174.9-175.5°C (EtOAc/hexane).

MS (CI⁺): *m/z* (%) = 447 (M+H ⁸¹Br, 100), 445 (M+H ⁷⁹Br, 92).

15 ¹H-NMR (DMSO d₆): δ = 3.71 (3H, s, CH₃), 3.73 (3H, s, CH₃), 3.76 (3H, s, CH₃), 4.22 (2H, d, J=5.8, CH₂), 6.62 (1H, t, J=5.7, NH), 6.80 (1H, m, ArH), 6.89 (2H, m, ArH), 6.98 (1H, m, ArH), 7.36-7.51 (3H, m, ArH), 7.63 (1H, s, ArH), 8.76 (1H, s, NH).

HPLC: retention time 2.86 min [Method A].

TLC: retention factor 0.20 (50% EtOAc/hexane).

20

Experiment 28

Preparation and Analysis of 116184

25 **{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(3,4,5-trimethoxyphenylmethyl)carboxamide**

[Protocol B] – 3,4,5-trimethoxybenzylamine

colourless solid (EtOAc/hexane).

MS (CI⁺): *m/z* (%) = 477 (M+H ⁸¹Br, 100), 475 (M+H ⁷⁹Br, 95).

30 ¹H-NMR (DMSO d₆): δ = 3.63 (3H, s, OCH₃), 3.75 (9H, s, 3xCH₃), 4.21 (1H, d, J=5.9, CH₂), 6.61 (2H, s, ArH), 6.65 (1H, t, J=5.9, NH), 6.99 (1H, m, ArH), 7.40 (1H, t, J=7.7, ArH), 7.45 (1H, m, ArH), 7.56 (1H, m, ArH), 7.64 (1H, s, ArH), 8.77 (1H, s, NH).

HPLC: retention time 5.91 min [Method B].

TLC: retention factor 0.50 (50% EtOAc/hexane).

Experiment 29

Preparation and Analysis of 116185

{{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(2-methylphenylmethyl)carboxamide

[Protocol B] – 2-methylbenzylamine

colourless solid (EtOAc/hexane).

MS (CI+): m/z (%) = 401 (M+H ^{81}Br , 96), 399 (M+H ^{79}Br , 100).

5 $^1\text{H-NMR}$ (DMSO d_6): δ = 2.28 (3H, s, CH_3), 3.76 (3H, s, NCH_3), 4.28 (1H, d, $J=5.8$, CH_2), 6.60 (1H, t, $J=5.8$, NH), 7.01 (1H, m, ArH), 7.15 (3H, m, ArH), 7.24 (1H, m, ArH), 7.38-7.50 (2H, m, ArH), 7.57 (1H, m, ArH), 7.65 (1H, s, ArH), 8.77 (1H, s, NH).

HPLC: retention time 2.74 min [Method A].

TLC: retention factor 0.20 (50% EtOAc/hexane).

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Experiment 30

Preparation and Analysis of 116189

{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-(4-methoxyphenylmethyl)carboxamide

15 [Protocol B] – 4-methoxybenzylamine

colourless solid (EtOAc/hexane).

MS (CI+): m/z (%) = 417 (M+H ^{81}Br , 94), 415 (M+H ^{79}Br , 100).

$^1\text{H-NMR}$ (DMSO d_6): δ = 3.72 (3H, s, CH_3), 3.77 (3H, s, NCH_3), 4.22 (1H, d, $J=5.9$, CH_2), 6.62 (1H, t, $J=5.9$, NH), 6.90 (2H, d, $J=8.8$, ArH), 7.00 (1H, m, ArH), 7.23
20 (2H, d, $J=8.8$, ArH), 7.39 (1H, t, $J=7.8$, ArH), 7.43 (1H, m, ArH), 7.56 (1H, m, ArH), 7.64 (1H, s, ArH), 8.73 (1H, s, NH).

HPLC: retention time 6.41 min [Method B].

TLC: retention factor 0.25 (50% EtOAc/hexane).

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Experiment 31

Preparation and Analysis of 116194

30 **{[3-(4-bromo-1-methylpyrazol-3-yl)phenyl]amino}-N-[2-(4-methoxy)phenylethyl]carboxamide**

[Protocol B] – 2-(4-methoxyphenyl)ethylamine

colourless solid (EtOAc/hexane).

MS (ES+): m/z (%) = 431 (M+H ^{81}Br , 95), 429 (M+H ^{79}Br , 100).

35 $^1\text{H-NMR}$ (DMSO d_6): δ = 2.68 (2H, t, $J=7.1$, CH_2), 3.31 (2H, m, CH_2), 3.71 (3H, s, CH_3), 3.77 (3H, s, CH_3), 6.16 (1H, t, $J=5.8$, NH), 6.87 (2H, d, $J=8.6$, ArH), 6.99 (1H, dt,

J=1.4, 7.3, ArH), 7.16 (2H, d, J=8.6, ArH), 7.33-7.48 (2H, m, ArH), 7.52 (1H, m, ArH), 7.63 (1H, s, ArH), 8.71 (1H, s, NH).

HPLC: retention time 6.62 min [Method B].

5 An important point that can be derived from the foregoing data is that by using a constitutively activated form of the receptor in the direct identification of candidate compounds, the selectivity of the compounds is exceptional: as those in the art appreciate, the homology between the human 5HT_{2A} and 5HT_{2C} receptors is about 95%, and even with such homology, certain of the directly identified compounds, e.g., 116081 and 116082 evidence a
10 100-fold difference in selectivity preference (as measured by IC₅₀ values) for the 5HT_{2A} receptor compared with the 5HT_{2C} receptor. This is important for pharmaceutical compositions in that such selectivity can help to reduce side-effects associated with interaction of a drug with a non-target receptor.

 Different embodiments of the invention will consist of different constitutively
15 activated receptors, different expression systems, different assays, and different compounds. Those skilled in the art will understand which receptors to use with which expression systems and assay methods. All are considered within the scope of the teaching of this invention. In addition, those skilled in the art will recognize that various modifications, additions, substitutions, and variations to the illustrative examples set forth herein can be made without
20 departing from the spirit of the invention and are, therefore, considered within the scope of the invention. All documents referenced above, including, but not limited to, provisional and regular patent applications, are fully incorporated herein by reference.

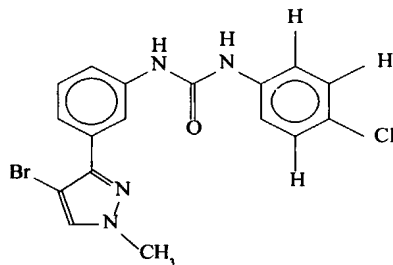
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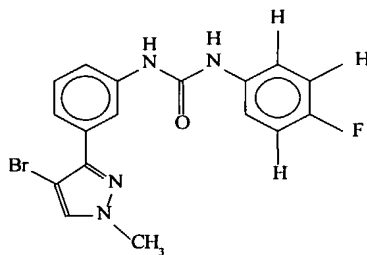
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CLAIMS**We claim:**

- 5 1. A compound structurally represented as follows:

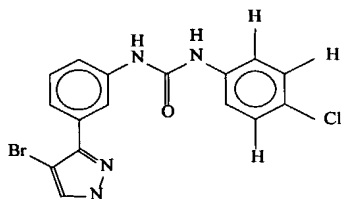


2. A compound structurally represented as follows:



10

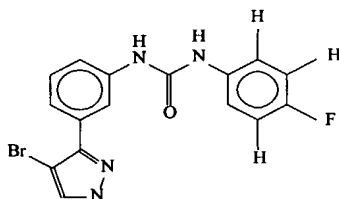
3. A composition comprising the compound of claim 1.
4. A composition comprising the compound of claim 2.
5. A compound structurally represented as follows:



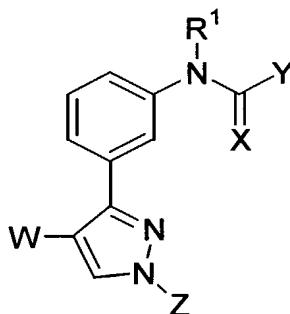
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6. A compound structurally represented as follows:



- 5 7. A composition comprising the compound of claim 5.
 8. A composition comprising the compound of claim 6.
 9. A compound structurally represented as follows:



10

wherein:

W is F, Cl, Br, I, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl;

15 **X** is O or S or NR²;

Y is NR³R⁴, or (CH₂)_mR⁵, or O(CH₂)_nR⁶

m is an integer between 0 and 4, inclusive;

n is an integer between 0 and 4, inclusive;

20 **Z** is H, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl;

R¹, R², R³ and R¹⁰ are each independently selected from: H, C₁₋₈ straight chain or branched alkyl, C₃₋₈ cycloalkyl, C₄₋₉ alkylcycloalkyl, or C₂₋₈ alkenyl;

R^4 , R^5 , and R^6 are each independently selected from: C_{1-8} straight chain or branched alkyl, C_{2-8} alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl, or CH_2 aryl, wherein each moiety within said C_{1-8} straight chain or branched alkyl, C_{2-8} alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH_2 aryl may be optionally substituted by up to four substituents in any position, whereby each substituent is independently selected from: H, F, Cl, Br, I, R^7 , CF_3 , CF_2R^7 , CF_2CF_2 , CCl_3 , CCl_2R^7 , $CCl_2CCl_2R^7$, NR^8R^9 , $NR^{10}COR^7$, $NR^{10}SO_2R^7$, OR^7 , OCF_3 , OCF_2R^7 , $OCF_2CF_2R^7$, $OCOR^7$, OSO_2R^7 , $OPO(OR^7)_2$, SR^7 , SCF_3 , SCF_2R^7 , $SCF_2CF_2R^7$, $SCOR^7$, SO_3R^7 , $SO_2NR^8R^9$, $PO(OR^7)_3$, $PO(OR^7)_2R^7$, NO_2 , CN , $CNR^{10}(NR^8R^9)$, $CNR^{10}(SR^7)$, $COOR^7$, $COSR^7$, $CONR^8R^9$,

with the proviso that when R^4 , R^5 , or R^6 contains an aryl ring substituted at at least two adjacent positions on said aryl ring, then said two adjacent positions can together be selected from SCH_2S , SCH_2CH_2S , OCH_2O , or OCH_2CH_2O to form a bi-cyclic structure;

R^7 is H, C_{1-8} straight chain or branched alkyl, C_{3-8} cycloalkyl, C_{4-9} alkylcycloalkyl, C_{2-8} alkenyl, aryl or alkylaryl; and

R^8 and R^9 are each independently selected from H, C_{1-8} straight chain or branched alkyl, C_{2-8} alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl, or CH_2 aryl, wherein each moiety within said C_{1-8} straight chain or branched alkyl, C_{2-8} alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH_2 aryl may be optionally substituted by up to four substituents in any position, whereby each substituent is independently selected from: F, Cl, Br, I, CF_3 , CCl_3 , CH_3 , C_2H_5 , C_3H_7 , C_4H_9 , NH_2 , $NHCH_3$, $N(CH_3)_2$, NHC_2H_5 , $N(C_2H_5)_2$, NHC_3H_7 , $N(C_3H_7)_2$, NHC_4H_9 , $N(C_4H_9)_2$, $NHCOH$, $NHCOCH_3$, $NHCOC_2H_5$, $NHCOC_3H_7$, $NHCOC_4H_9$, $NHSO_2CH_3$, $NHSO_2C_2H_5$, $NHSO_2C_3H_7$, $NHSO_2C_4H_9$, OH , OCH_3 , OC_2H_5 , OC_3H_7 , OC_4H_7 , OC_4H_9 , OC_5H_9 , OC_5H_{11} , OC_6H_{11} , OC_6H_{13} , OCF_3 , $OCOCH_3$, $OCOC_2H_5$, $OCOC_3H_7$, $OCOC_4H_9$, OSO_2CH_3 , $OSO_2C_2H_5$, $OSO_2C_3H_7$, $OSO_2C_4H_9$, SH , SCH_3 , SC_2H_5 , SC_3H_7 , SC_4H_7 , SC_4H_9 , SC_5H_9 , SC_5H_{11} , SC_6H_{11} , SC_6H_{13} , SCF_3 , $SCOCH_3$, $SCOC_2H_5$, $SCOC_3H_7$, $SCOC_4H_9$, SO_3CH_3 , $SO_3C_2H_5$, $SO_3C_3H_7$, $SO_3C_4H_9$, SO_2NH , SO_2NH_2 , SO_2NHCH_3 , $SO_2N(CH_3)_2$, $SO_2NHC_2H_5$, $SO_2N(C_2H_5)_2$, $SO_2NHC_3H_7$, $SO_2N(C_3H_7)_2$, $SO_2NHC_4H_9$, $SO_2N(C_4H_9)_2$, NO_2 , CN , $COOCH_3$, $COOC_2H_5$, $COOC_3H_7$, $COOC_4H_9$, $COSCH_3$, $COSC_2H_5$, $COSC_3H_7$, $COSC_4H_9$, $CONH_2$, $CONHCH_3$, $CON(CH_3)_2$, $CONHC_2H_5$, $CON(C_2H_5)_2$, $CONHC_3H_7$, $CON(C_3H_7)_2$, $CONHC_4H_9$, $CON(C_4H_9)_2$, and

with the proviso that when either or both of R^8 or R^9 contain an aryl ring substituted at at least two adjacent positions on said aryl ring, then said two

adjacent positions can together be selected from SCH₂S, SCH₂CH₂S, OCH₂O, or OCH₂CH₂O to form a bi-cyclic structure;

or

R⁸ and **R**⁹ may together form part of a 5, 6 or 7 membered cyclic structure, with
 5 said structure being saturated or unsaturated, and further with said structure containing up
 to four heteroatoms selected from O, N or S, and further wherein each moiety within said
 cyclic structure being optionally substituted by up to four substituents in any position
 independently selected from: F, Cl, Br, I, CF₃, CCl₃, CH₃, C₂H₅, C₃H₇, C₄H₉, NH₂,
 NHCH₃, N(CH₃)₂, NHC₂H₅, N(C₂H₅)₂, NHC₃H₇, N(C₃H₇)₂, NHC₄H₉, N(C₄H₉)₂, NHCOH,
 10 NHCOCH₃, NHCOC₂H₅, NHCOC₃H₇, NHCOC₄H₉, NHSO₂CH₃, NHSO₂C₂H₅,
 NHSO₂C₃H₇, NHSO₂C₄H₉, OH, OCH₃, OC₂H₅, OC₃H₇, OC₄H₇, OC₄H₉, OC₅H₉, OC₅H₁₁,
 OC₆H₁₁, OC₆H₁₃, OCF₃, OCOCH₃, OCOC₂H₅, OCOC₃H₇, OCOC₄H₉, OSO₂CH₃,
 OSO₂C₂H₅, OSO₂C₃H₇, OSO₂C₄H₉, SH, SCH₃, SC₂H₅, SC₃H₇, SC₄H₇, SC₄H₉, SC₅H₉,
 SC₅H₁₁, SC₆H₁₁, SC₆H₁₃, SCF₃, SCOCH₃, SCOC₂H₅, SCOC₃H₇, SCOC₄H₉, SO₃CH₃,
 15 SO₃C₂H₅, SO₃C₃H₇, SO₃C₄H₉, SO₂NH, SO₂NH₂, SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅,
 SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂, SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN,
 COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉, COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉,
 CONH₂, CONHCH₃, CON(CH₃)₂, CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂,
 CONHC₄H₉, CON(C₄H₉)₂, and

20 with the proviso that wherein when **R**⁸ and **R**⁹ form an aryl ring substituted
 at at least two adjacent positions on said aryl ring, then said two adjacent
 positions can together be selected from SCH₂S, SCH₂CH₂S, OCH₂O, or
 OCH₂CH₂O to form a bi-cyclic structure.

10. A composition comprising a compound of claim 9.

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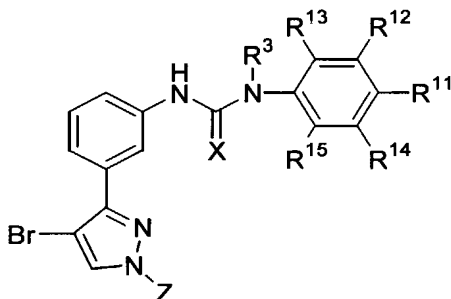
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11. A compound structurally represented as follows:



5 wherein:

$X = O$ or S ;

Z is H or CH_3 ;

R^3 and R^{10} are each independently selected from H , C_{1-8} straight chain or branched alkyl, C_{3-8} cycloalkyl, C_{4-9} alkylcycloalkyl, or C_{2-8} alkenyl;

R^{11} is H , F , Cl , Br , I , R^7 , CF_3 , CCl_3 , NR^8R^9 , $NR^{10}COR^7$, $NR^{10}SO_2R^7$, OR^7 , OCF_3 , $OCOR^7$, OSO_2R^7 , SR^7 , SCF_3 , $SCOR^7$, SO_3R^7 , $SO_2NR^8R^9$, NO_2 , CN , $COOR^7$, $COSR^7$, $CONR^8R^9$,

with the proviso that when a position adjacent to R^{11} is substituted, then R^{11} and said adjacent position can together be selected from SCH_2S , SCH_2CH_2S , OCH_2O , or OCH_2CH_2O to form a bi-cyclic structure;

R^7 is H , C_{1-8} straight chain or branched alkyl, C_{3-8} cycloalkyl, C_{4-9} alkylcycloalkyl, C_{2-8} alkenyl, aryl or alkylaryl;

R^8 and R^9 are independently selected from H , C_{1-8} straight chain or branched alkyl, C_{2-8} alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH_2 aryl wherein each moiety within said C_{1-8} straight chain or branched alkyl, C_{2-8} alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH_2 aryl may be optionally substituted by up to four substituents in any position, whereby each substituent is independently selected from: F , Cl , Br , I , CF_3 , CCl_3 , CH_3 , C_2H_5 , C_3H_7 , C_4H_9 , NH_2 , $NHCH_3$, $N(CH_3)_2$, NHC_2H_5 , $N(C_2H_5)_2$, NHC_3H_7 , $N(C_3H_7)_2$, NHC_4H_9 , $N(C_4H_9)_2$, $NHCOH$, $NHCOCH_3$, $NHCOC_2H_5$, $NHCOC_3H_7$, $NHCOC_4H_9$, $NHSO_2CH_3$, $NHSO_2C_2H_5$, $NHSO_2C_3H_7$, $NHSO_2C_4H_9$, OH , OCH_3 , OC_2H_5 , OC_3H_7 , OC_4H_9 , OC_5H_9 , OC_5H_{11} , OC_6H_{11} , OC_6H_{13} , OCF_3 , $OCOCH_3$, $OCOC_2H_5$,

OCOC₃H₇, OCOC₄H₉, OSO₂CH₃, OSO₂C₂H₅, OSO₂C₃H₇, OSO₂C₄H₉, SH, SCH₃, SC₂H₅, SC₃H₇, SC₄H₇, SC₄H₉, SC₅H₉, SC₅H₁₁, SC₆H₁₁, SC₆H₁₃, SCF₃, SCOCH₃, SCOC₂H₅, SCOC₃H₇, SCOC₄H₉, SO₃CH₃, SO₃C₂H₅, SO₃C₃H₇, SO₃C₄H₉, SO₂NH, SO₂NH₂, SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅, SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂,
 5 SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN, COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉, COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉, CONH₂, CONHCH₃, CON(CH₃)₂, CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂, CONHC₄H₉, CON(C₄H₉)₂, and

with the proviso that when either of **R**⁸ or **R**⁹ contain an aryl ring substituted at at least two adjacent positions on said aryl ring, then said two
 10 adjacent positions can together be selected from SCH₂S, SCH₂CH₂S, OCH₂O, or OCH₂CH₂O to form a bi-cyclic structure;

R¹², **R**¹³, **R**¹⁴, and **R**¹⁵ are each independently selected from: F, Cl, Br, I, CF₃, CCl₃, CH₃, C₂H₅, C₃H₇, C₄H₉, NH₂, NHCH₃, N(CH₃)₂, NHC₂H₅, N(C₂H₅)₂, NHC₃H₇, N(C₃H₇)₂, NHC₄H₉, N(C₄H₉)₂, NHCOH, NHCOCH₃, NHCOC₂H₅, NHCOC₃H₇, NHCOC₄H₉,
 15 NHSO₂CH₃, NHSO₂C₂H₅, NHSO₂C₃H₇, NHSO₂C₄H₉, OH, OCH₃, OC₂H₅, OC₃H₇, OC₄H₇, OC₄H₉, OC₅H₉, OC₅H₁₁, OC₆H₁₁, OC₆H₁₃, OCF₃, OCOCH₃, OCOC₂H₅, OCOC₃H₇, OCOC₄H₉, OSO₂CH₃, OSO₂C₂H₅, OSO₂C₃H₇, OSO₂C₄H₉, SH, SCH₃, SC₂H₅, SC₃H₇, SC₄H₇, SC₄H₉, SC₅H₉, SC₅H₁₁, SC₆H₁₁, SC₆H₁₃, SCF₃, SCOCH₃, SCOC₂H₅, SCOC₃H₇, SCOC₄H₉, SO₃CH₃, SO₃C₂H₅, SO₃C₃H₇, SO₃C₄H₉, SO₂NH, SO₂NH₂,
 20 SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅, SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂, SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN, COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉, COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉, CONH₂, CONHCH₃, CON(CH₃)₂, CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂, CONHC₄H₉, CON(C₄H₉)₂, and

with the proviso that when any two adjacent positions of **R**¹², **R**¹³, **R**¹⁴, and
 25 **R**¹⁵ are substituted, said two adjacent positions can together be further selected from SCH₂S, SCH₂CH₂S, OCH₂O, or OCH₂CH₂O to form a bi-cyclic structure; and

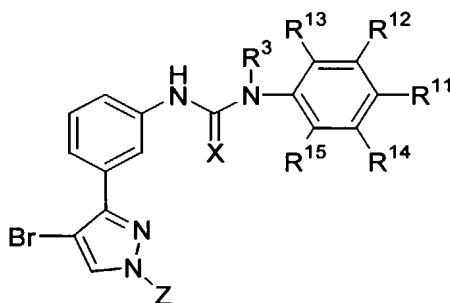
with the proviso that at least two of **R**¹², **R**¹³, **R**¹⁴, and **R**¹⁵ must be H.

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12. The compound of claim 11 structurally represented as follows:



5 wherein:

$X = O$ or S ;

Z is H or CH_3 ;

R^3 and R^{10} are each independently selected from H , C_{1-8} straight chain or branched
10 alkyl, C_{3-8} cycloalkyl, C_{4-9} alkylcycloalkyl, or C_{2-8} alkenyl;

R^{11} is H , F , Cl , Br , I , R^7 , CF_3 , CCl_3 , NR^8R^9 , $NR^{10}COR^7$, $NR^{10}SO_2R^7$, OR^7 , OCF_3 ,
 $OCOR^7$, OSO_2R^7 , SR^7 , SCF_3 , $SCOR^7$, SO_3R^7 , $SO_2NR^8R^9$, NO_2 , CN , $COOR^7$, $COSR^7$,
 $CONR^8R^9$,

with the proviso that when a position adjacent to R^{11} is substituted, then R^{11}
15 and said adjacent position can together be selected from SCH_2S ,
 SCH_2CH_2S , OCH_2O , or OCH_2CH_2O to form a bi-cyclic structure;

R^7 is H , C_{1-8} straight chain or branched alkyl, C_{3-8} cycloalkyl, C_{4-9} alkylcycloalkyl,
 C_{2-8} alkenyl, aryl or alkylaryl;

R^8 and R^9 are independently selected from H , C_{1-8} straight chain or branched alkyl,
20 C_{2-8} alkenyl or cycloalkyl, or alkylcycloalkyl, or aryl or CH_2 aryl wherein each moiety
within said C_{1-8} straight chain or branched alkyl, C_{2-8} alkenyl or cycloalkyl, or
alkylcycloalkyl, or aryl or CH_2 aryl may be optionally substituted by up to four substituents
in any position, whereby each substituent is independently selected from: F , Cl , Br , I , CF_3 ,
 CCl_3 , CH_3 , C_2H_5 , C_3H_7 , C_4H_9 , NH_2 , $NHCH_3$, $N(CH_3)_2$, NHC_2H_5 , $N(C_2H_5)_2$, NHC_3H_7 ,
25 $N(C_3H_7)_2$, NHC_4H_9 , $N(C_4H_9)_2$, $NHCOH$, $NHCOCH_3$, $NHCOC_2H_5$, $NHCOC_3H_7$,
 $NHCOC_4H_9$, $NHSO_2CH_3$, $NHSO_2C_2H_5$, $NHSO_2C_3H_7$, $NHSO_2C_4H_9$, OH , OCH_3 , OC_2H_5 ,
 OC_3H_7 , OC_4H_9 , OC_5H_{11} , OC_6H_{13} , OCF_3 , $OCOCH_3$, $OCOC_2H_5$,

OCOC₃H₇, OCOC₄H₉, OSO₂CH₃, OSO₂C₂H₅, OSO₂C₃H₇, OSO₂C₄H₉, SH, SCH₃, SC₂H₅, SC₃H₇, SC₄H₇, SC₄H₉, SC₅H₉, SC₅H₁₁, SC₆H₁₁, SC₆H₁₃, SCF₃, SCOCH₃, SCOC₂H₅, SCOC₃H₇, SCOC₄H₉, SO₃CH₃, SO₃C₂H₅, SO₃C₃H₇, SO₃C₄H₉, SO₂NH, SO₂NH₂, SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅, SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂,
 5 SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN, COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉, COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉, CONH₂, CONHCH₃, CON(CH₃)₂, CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂, CONHC₄H₉, CON(C₄H₉)₂, and

with the proviso that when either of **R**⁸ or **R**⁹ contain an aryl ring substituted at at least two adjacent positions on said aryl ring, then said two adjacent positions can together be selected from SCH₂S, SCH₂CH₂S,
 10 OCH₂O, or OCH₂CH₂O to form a bi-cyclic structure;

R¹², **R**¹³, **R**¹⁴, and **R**¹⁵ are each independently selected from: F, Cl, Br, I, CF₃, CCl₃, CH₃, C₂H₅, C₃H₇, C₄H₉, NH₂, NHCH₃, N(CH₃)₂, NHC₂H₅, N(C₂H₅)₂, NHC₃H₇, N(C₃H₇)₂, NHC₄H₉, N(C₄H₉)₂, NHCOH, NHCOCH₃, NHCOC₂H₅, NHCOC₃H₇, NHCOC₄H₉,
 15 NHSO₂CH₃, NHSO₂C₂H₅, NHSO₂C₃H₇, NHSO₂C₄H₉, OH, OCH₃, OC₂H₅, OC₃H₇, OC₄H₇, OC₄H₉, OC₅H₉, OC₅H₁₁, OC₆H₁₁, OC₆H₁₃, OCF₃, OCOCH₃, OCOC₂H₅, OCOC₃H₇, OCOC₄H₉, OSO₂CH₃, OSO₂C₂H₅, OSO₂C₃H₇, OSO₂C₄H₉, SH, SCH₃, SC₂H₅, SC₃H₇, SC₄H₇, SC₄H₉, SC₅H₉, SC₅H₁₁, SC₆H₁₁, SC₆H₁₃, SCF₃, SCOCH₃, SCOC₂H₅, SCOC₃H₇, SCOC₄H₉, SO₃CH₃, SO₃C₂H₅, SO₃C₃H₇, SO₃C₄H₉, SO₂NH, SO₂NH₂,
 20 SO₂NHCH₃, SO₂N(CH₃)₂, SO₂NHC₂H₅, SO₂N(C₂H₅)₂, SO₂NHC₃H₇, SO₂N(C₃H₇)₂, SO₂NHC₄H₉, SO₂N(C₄H₉)₂, NO₂, CN, COOCH₃, COOC₂H₅, COOC₃H₇, COOC₄H₉, COSCH₃, COSC₂H₅, COSC₃H₇, COSC₄H₉, CONH₂, CONHCH₃, CON(CH₃)₂, CONHC₂H₅, CON(C₂H₅)₂, CONHC₃H₇, CON(C₃H₇)₂, CONHC₄H₉, CON(C₄H₉)₂, and

with the proviso that when any two adjacent positions of **R**¹², **R**¹³, **R**¹⁴, and **R**¹⁵ are substituted, said two adjacent positions can together be further selected from SCH₂S, SCH₂CH₂S, OCH₂O, or OCH₂CH₂O to form a bi-cyclic structure; and
 25

with the proviso that at least two of **R**¹², **R**¹³, **R**¹⁴, and **R**¹⁵ must be H.

13. A composition comprising a compound of claim 11.

30 14. A composition comprising a compound of claim 12.

//

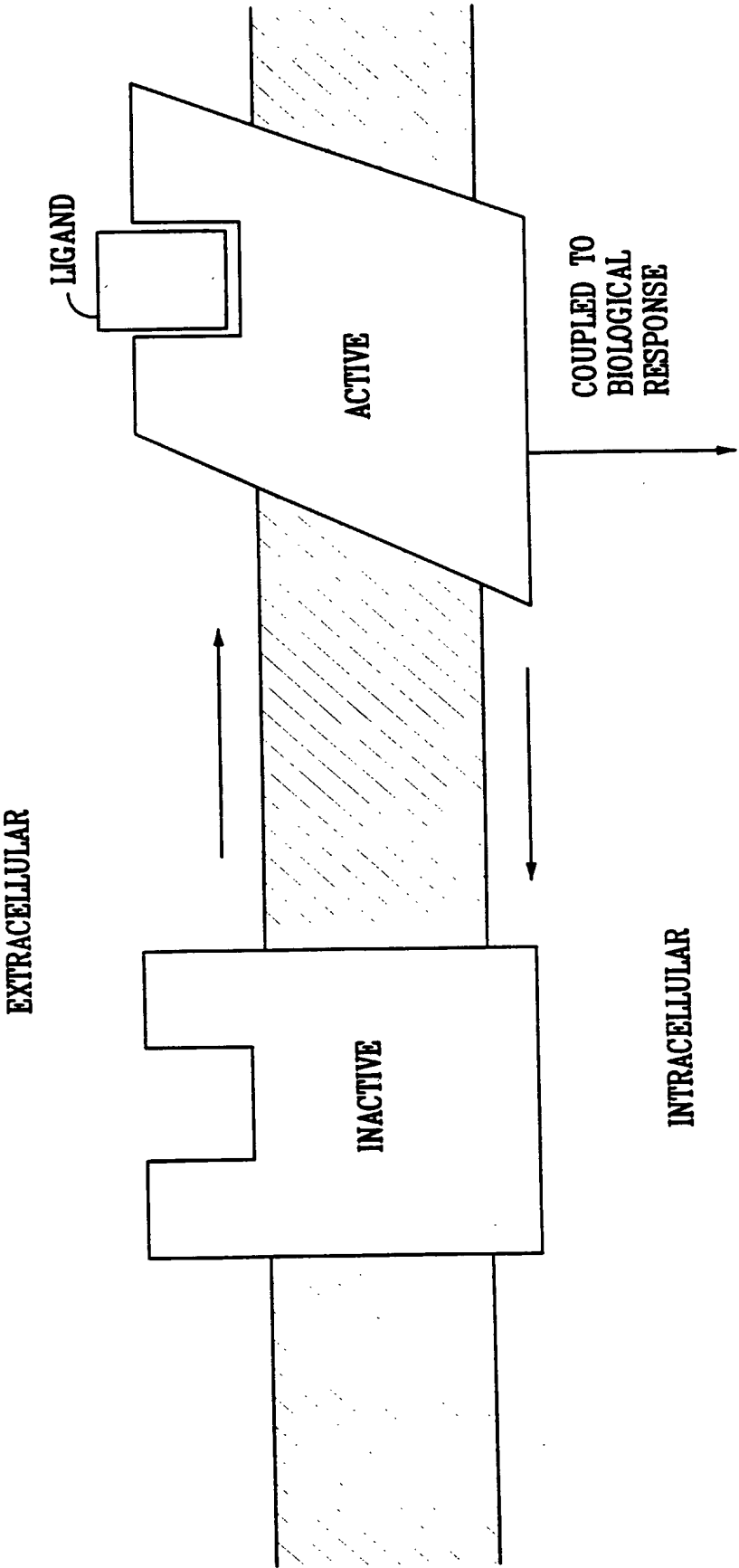


FIG. 1

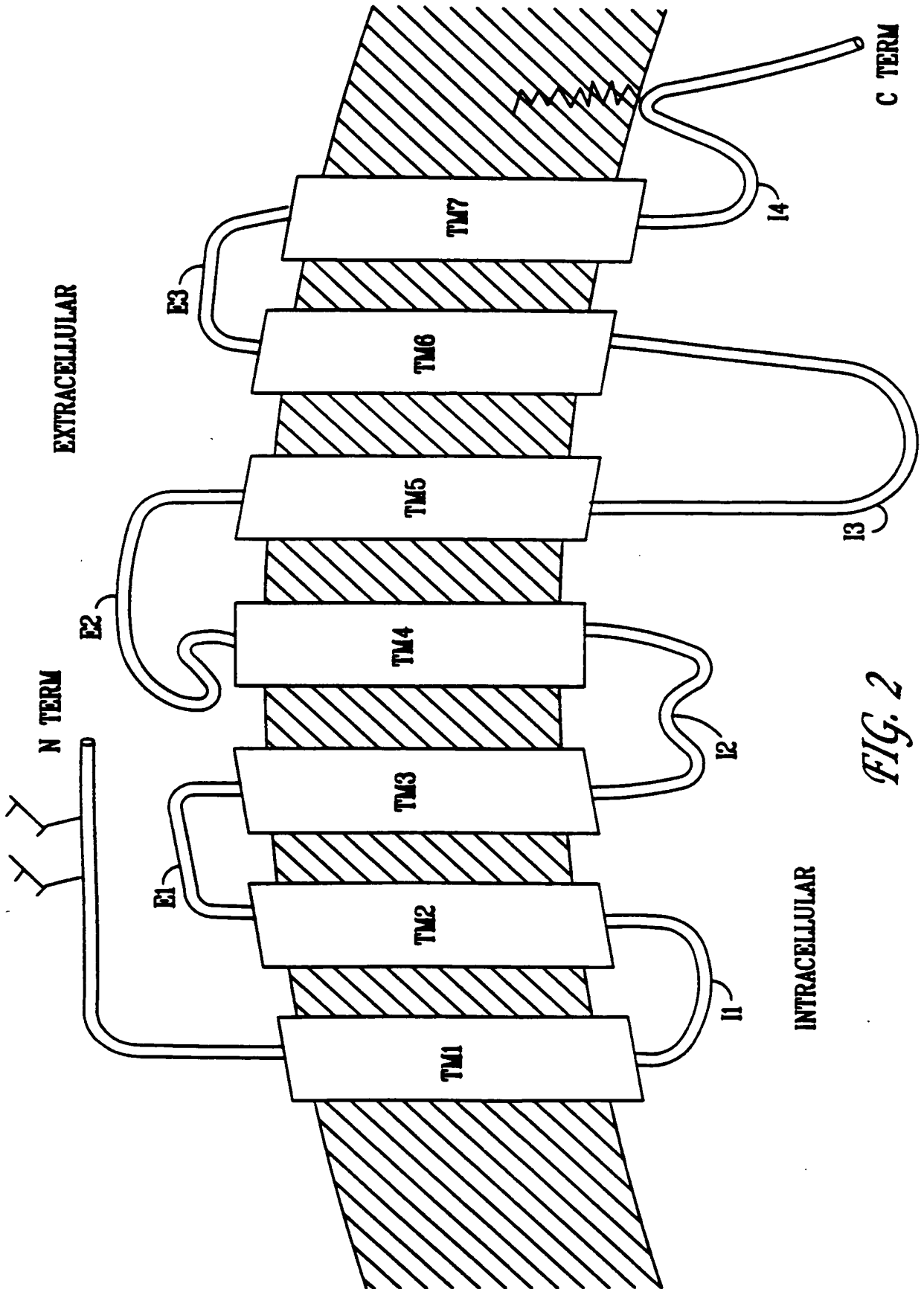


Figure 3

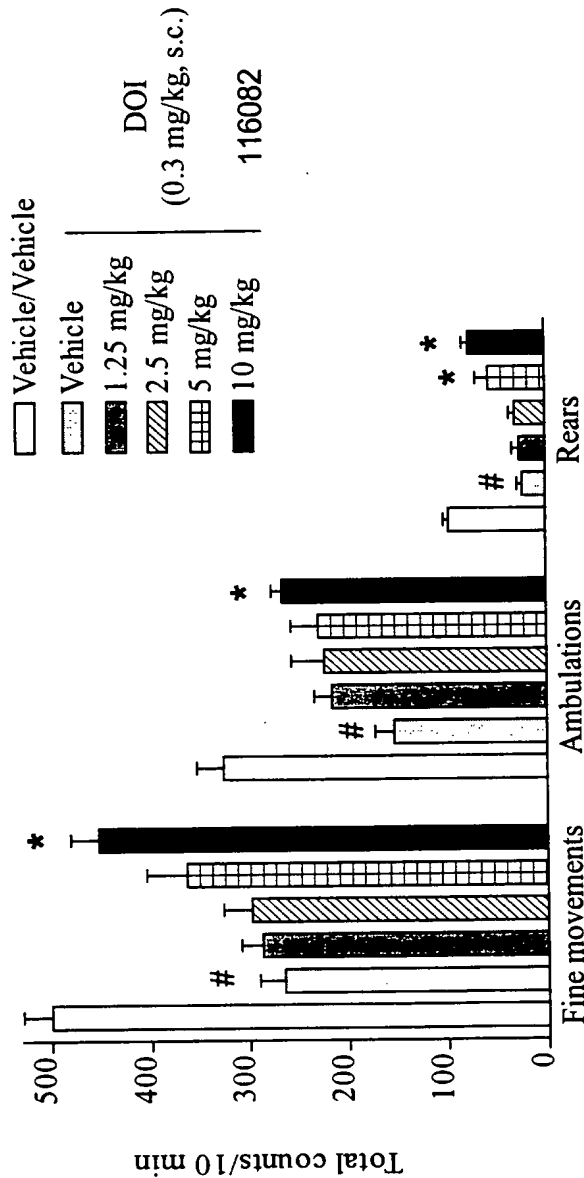


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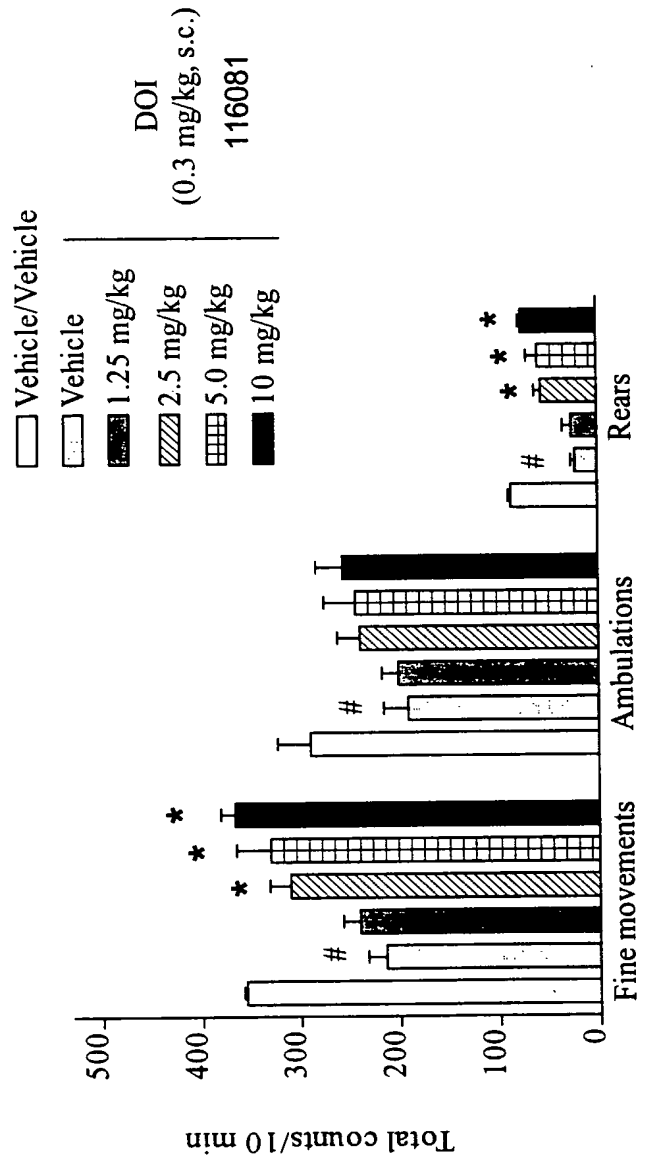


Figure 5

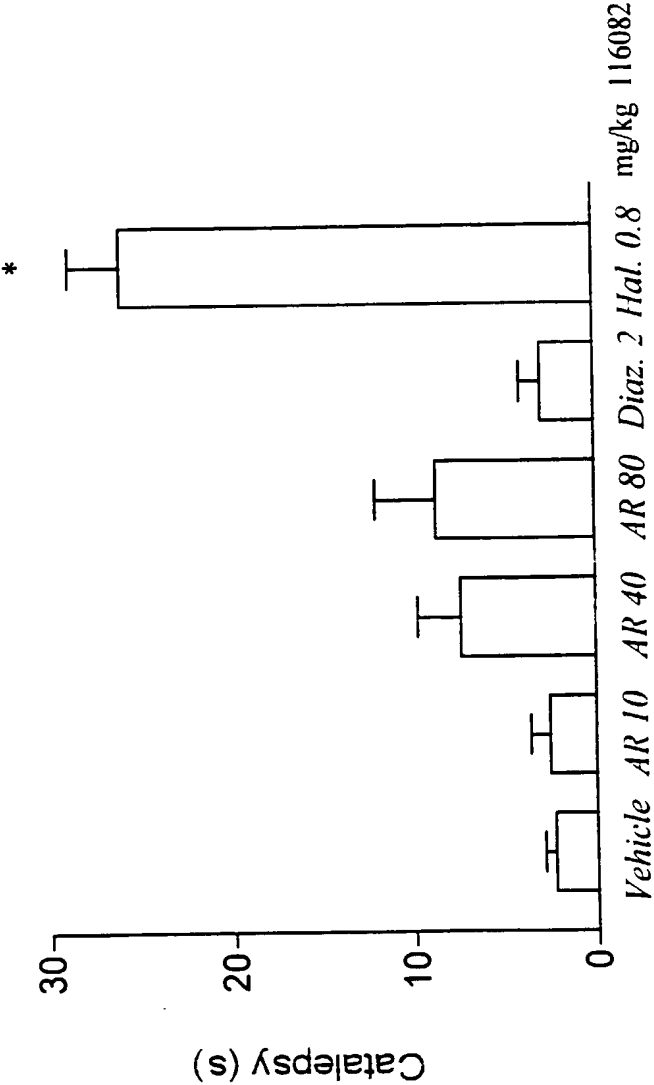


Figure 6

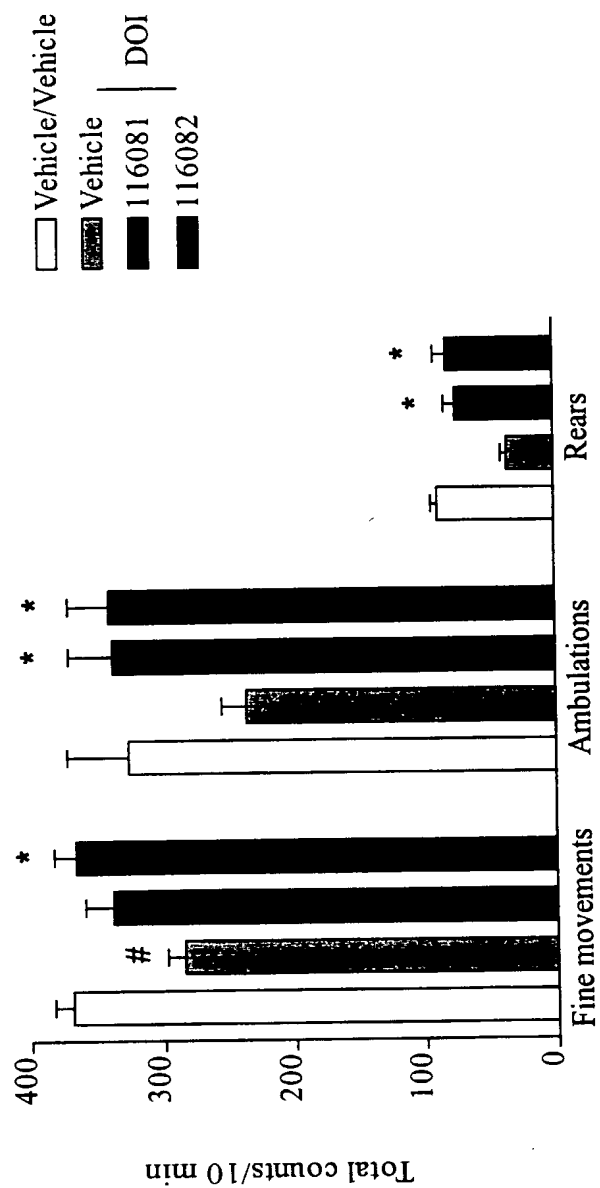
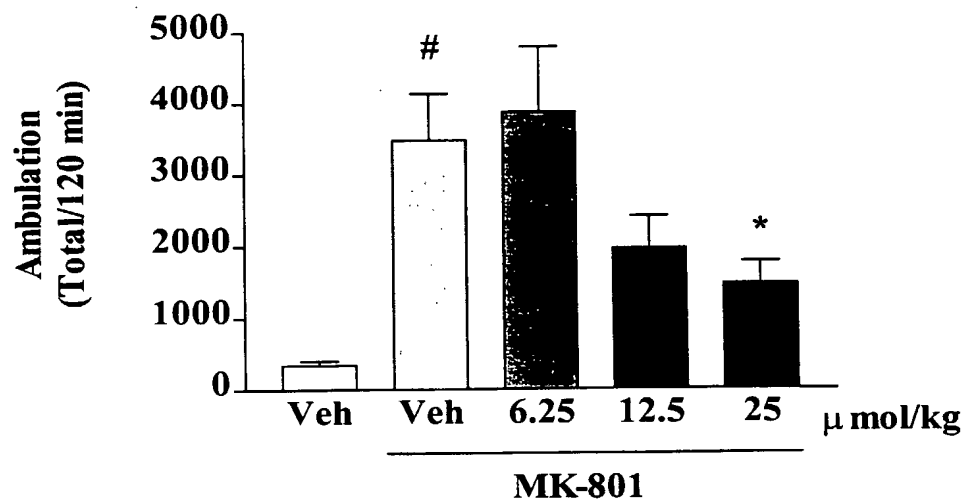
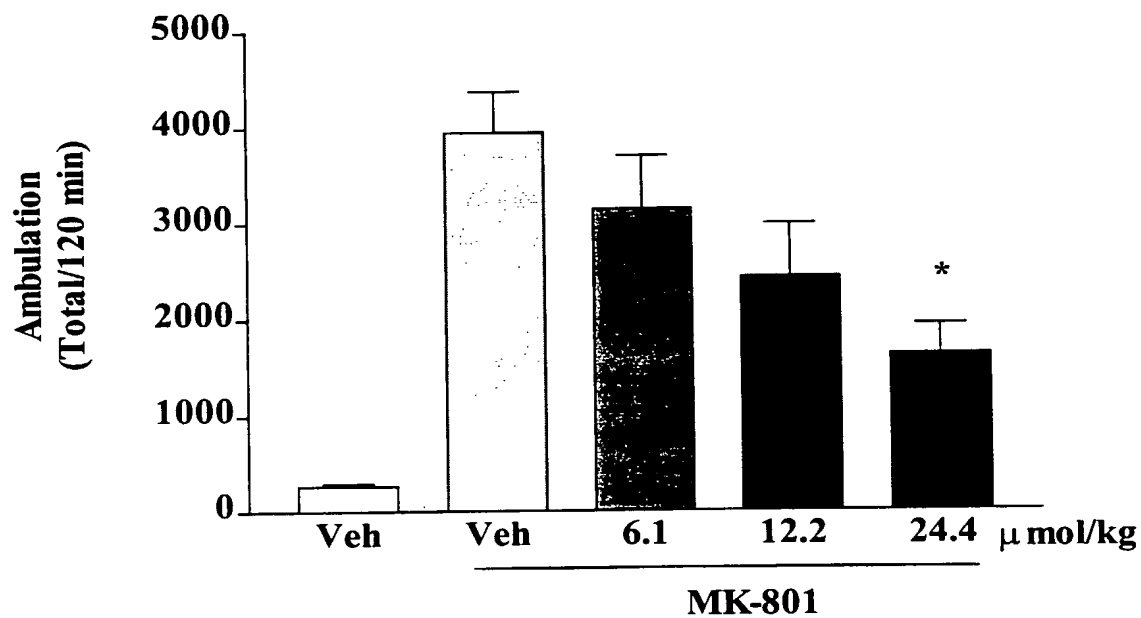


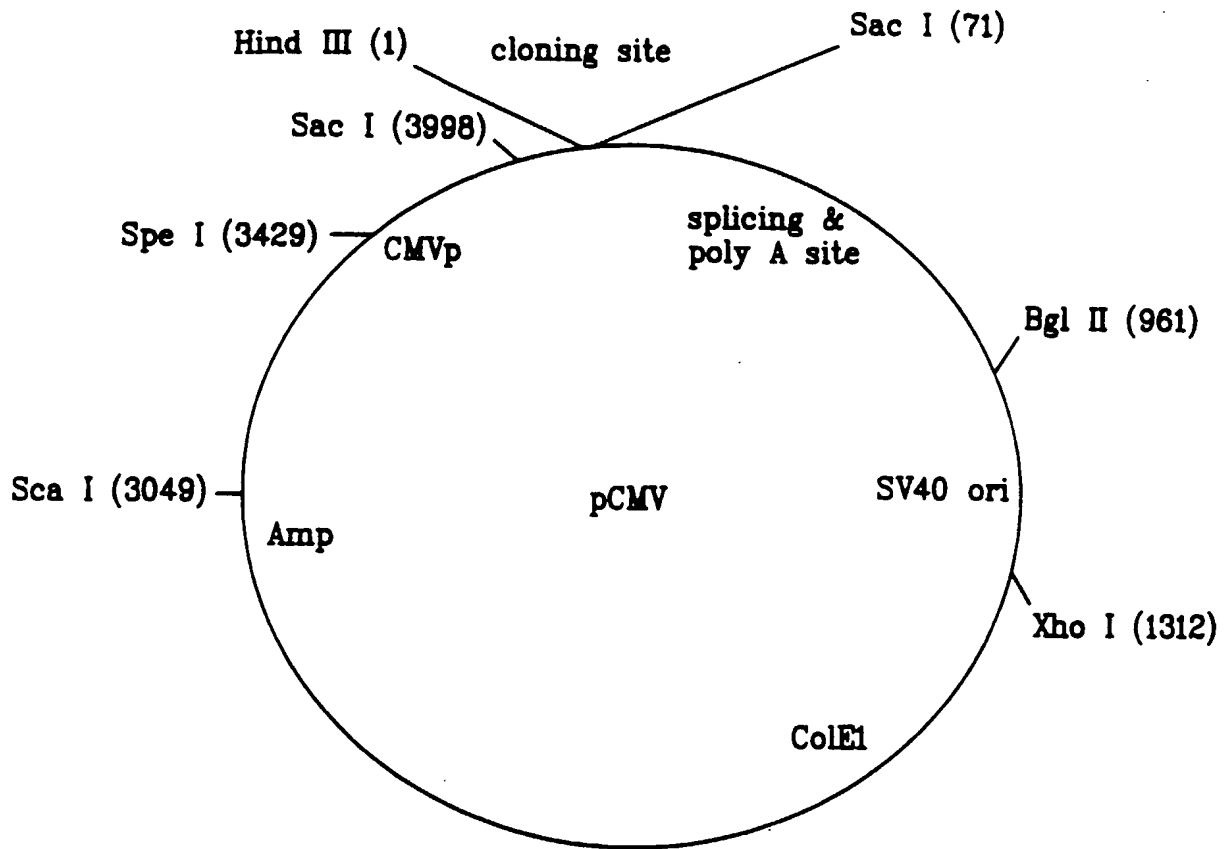
Figure 7

7A-116081



7B-CLOZAPINE





*Xho I (1312) to Sca I (3049) is identical to pRc/RSV Xho I (3045) to 4782.

*Sca I (3049) to 4070 is identical to pCDM7 Amp Scal (2524) to 3545.

*multiple cloning site includes Hind III to Sac I of pBluescript II.

*110 to 1312 is identical to pCMD7 Amp 76 to 1278.

*Sac I and Spe I in MCS are not unique.

FIG. 8

SEQUENCE LISTING

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 65 70 75 80
 Glu Lys Lys Leu His Asn Ala Thr Asn Tyr Phe Leu Met Ser Leu Ala
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 130 135 140
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Cys Leu Ser Leu Leu His Leu Gln Glu Lys Asn Trp Ser Ala Leu Leu
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| Cys | Leu | Ser | Leu | Leu | His | Leu | Gln | Glu | Lys | Asn | Trp | Ser | Ala | Leu | Leu |
| 65 | | | | | 70 | | | | | 75 | | | | | 80 |
| Thr | Ala | Val | Val | Ile | Ile | Leu | Thr | Ile | Ala | Gly | Asn | Ile | Leu | Val | Ile |
| | | | | 85 | | | | | 90 | | | | | 95 | |
| Met | Ala | Val | Ser | Leu | Glu | Lys | Lys | Leu | Gln | Asn | Ala | Thr | Asn | Tyr | Phe |
| | | | 100 | | | | | 105 | | | | | 110 | | |
| Leu | Met | Ser | Leu | Ala | Ile | Ala | Asp | Met | Leu | Leu | Gly | Phe | Leu | Val | Met |
| | | 115 | | | | | 120 | | | | | 125 | | | |
| Pro | Val | Ser | Met | Leu | Thr | Ile | Leu | Tyr | Gly | Tyr | Arg | Trp | Pro | Leu | Pro |
| | 130 | | | | | 135 | | | | | 140 | | | | |

Ser Lys Leu Cys Ala Val Trp Ile Tyr Leu Asp Val Leu Phe Ser Thr
 145 150 155 160
 Ala Ser Ile Met His Leu Cys Ala Ile Ser Leu Asp Arg Tyr Val Ala
 165 170 175
 Ile Gln Asn Pro Ile His His Ser Arg Phe Asn Ser Arg Thr Lys Ala
 180 185 190
 Phe Leu Lys Ile Ile Ala Val Trp Thr Ile Ser Val Gly Ile Ser Met
 195 200 205
 Pro Ile Pro Val Phe Gly Leu Gln Asp Asp Ser Lys Val Phe Lys Glu
 210 215 220
 Gly Ser Cys Leu Leu Ala Asp Asp Asn Phe Val Leu Ile Gly Ser Phe
 225 230 235 240
 Val Ser Phe Phe Ile Pro Leu Thr Ile Met Val Ile Thr Tyr Cys Leu
 245 250 255
 Thr Ile Tyr Val Leu Arg Arg Gln Ala Leu Met Leu Leu His Gly His
 260 265 270
 Thr Glu Glu Pro Pro Gly Leu Ser Leu Asp Phe Leu Lys Cys Cys Lys
 275 280 285
 Arg Asn Thr Ala Glu Glu Glu Asn Ser Ala Asn Pro Asn Gln Asp Gln
 290 295 300
 Asn Ala Arg Arg Arg Lys Lys Lys Glu Arg Arg Pro Arg Gly Thr Met
 305 310 315 320
 Gln Ala Ile Asn Asn Glu Arg Lys Ala Lys Lys Val Leu Gly Ile Val
 325 330 335
 Phe Phe Val Phe Leu Ile Met Trp Cys Pro Phe Phe Ile Thr Asn Ile
 340 345 350
 Met Ala Val Ile Cys Lys Glu Ser Cys Asn Glu Asp Val Ile Gly Ala
 355 360 365
 Leu Leu Asn Val Phe Val Trp Ile Gly Tyr Leu Ser Ser Ala Val Asn
 370 375 380
 Pro Leu Val Tyr Thr Leu Phe Asn Lys Ile Tyr Arg Arg Ala Phe Ser
 385 390 395 400
 Asn Tyr Leu Arg Cys Asn Tyr Lys Val Glu Lys Lys Pro Pro Val Arg
 405 410 415

Gln Ile Pro Arg Val Ala Ala Thr Ala Leu Ser Gly Arg Glu Leu Asn
420 425 430

Val Asn Ile Tyr Arg His Thr Asn Glu Pro Val Ile Glu Lys Ala Ser
435 440 445

Asp Asn Glu Pro Gly Ile Glu Met Gln Val Glu Asn Leu Glu Leu Pro
450 455 460

Val Asn Pro Ser Ser Val Val Ser Glu Arg Ile Ser Ser Val
465 470 475

INTERNATIONAL SEARCH REPORT

Int. l. Application No

PCT/US 00/28347

A. CLASSIFICATION OF SUBJECT MATTER
 IPC 7 C07D231/16 A61P25/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|-----------------------|
| P, X | WO 99 52927 A (SMITH JULIAN R ; FOSTER RICHARD J (GB); ARENA PHARMACEUTICALS INC () 21 October 1999 (1999-10-21) cited in the application page 36 -page 41; claims 15-19 ----- | 1-14 |
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☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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P document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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Date of the actual completion of the international search

31 January 2001

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/28347

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